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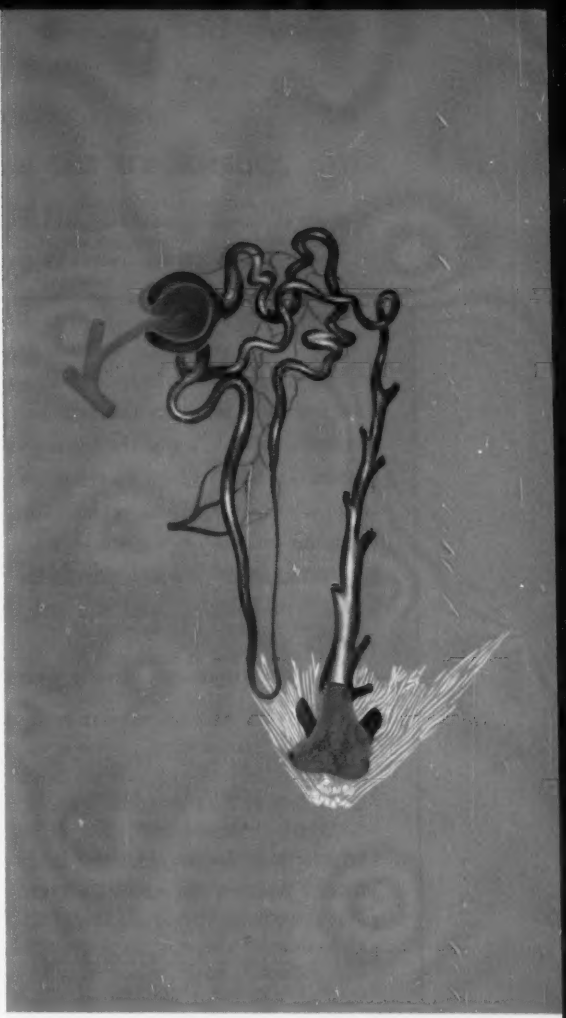

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JOHN F. FULTON (1899-1960)

A Midsummer Reminiscence

In June of 1957 word came to me in Zürich that Dr. Fulton was gravely ill. He had recently arrived in London to celebrate the Harvey Tercentenary and was to go on from there to Oxford to receive an honorary degree. He had rallied from critical illnesses before, but this time it seemed that the end had come. I boarded a plane for London.

The muffled roar of the all-but-empty plane and the looming into view of the snow-covered Alps were conducive to reverie. Successively, the Finsteraarhorn, the dour north face of the Eiger, the Mönch, the Jungfrau, and faraway Mont Blanc caught the mist of one's thoughts and caused it to linger over the little town of Nyon, Switzerland. Noviodunum. Here on the northern shore of Lake Geneva was Les Terrasses, the villa of Arnold Klebs, the great friend of Fulton and Harvey Cushing. Here young Fulton and Cushing had been inspired by an incomparable library of incunabula. Here had been the starting place of expeditions over Simplon Pass to Italy —Ravenna, Milan, Bologna—in search of rare books. Here possibly had begun an expedition in the minds of the three men to reach the peak of excellence in the form of an historical library which would house their respective collections, an expedition that culminated in the achievement of the Yale Medical Historical Library.

The plane continued its course over France, another country which Dr. Fulton loved, and which reciprocated his affection and admiration by making him an officer in the Legion of Honor. Thereafter he was never seen without the insignia—the small red rosette—in his lapel. Today it seemed that he would not live to receive the honor that would probably have meant more than any—the degree from Oxford. *La vie et la mort.* Out of the patchwork of green and orange plowed fields lying three miles below grew the image of Bichat, the young French physiologist of the last century, who like Fulton had achieved an early fame, and who, just before his untimely

* Chief, Section on Limbic Integration and Behavior.

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death at the age of 31, had published his treatise, *Recherches physiologiques sur la vie et la mort.*

History will possibly contrast the contributions of the two men. Bichat divided the nerves of the body into two great systems—one, the cerebrospinal (or voluntary) nervous system arising in the brain; the other, the ganglionic nervous system, taking origin in the ganglia. He referred to the ganglia as "little brains" and argued that they and the viscera which they innervated were the source of emotions.

In the physical science that grew out of the discoveries of Galileo, man could hope for increasing emancipation from the vicissitudes of his environment. In the American and French Revolutions, he could see the promise of breaking everywhere the chains that bind man to man. But owing to the persuasive appeal of Bichat's two independently functioning nervous systems, it was to be more than one hundred years before man could dare to hope that he might partially free himself from the ruling passions within. Freud was so immersed in Bichat's doctrine that he believed visceral symptoms and affections called psychosomatic today could not be psychological in origin.

The Bichat doctrine received its first mortal blow from Gaskell and Langley. Thereafter, as the result of the work of Karplus, Kreidl, Hess, Cannon, Bard, and others, the emotions and the viscera slowly worked their way up the neuraxis to find representation as high as the level of the diencephalon in the so-called voluntary nervous system. As late as 1936 Cannon authoritatively stated that the cortex was concerned with emotion only insofar as it could inhibit those aspects of emotion under voluntary control. It was of major consequence that at that time Fulton and his colleagues were already demonstrating in the Yale Laboratory that parts of the cortex were actively engaged in the elaboration of emotion and visceral manifestations.

Switzerland. Italy. Belgium. France. Dr. Fulton belonged to all of these, and he belonged so much to the green island now visible across the Channel that some of his compatriots upbraided him for being an Anglophile. But in a sense Fulton belonged to all countries—a true Goldsmithian Citizen of the World—just as, in a sense, he belonged to all times. In reading, writing, speaking, he "inviscerated" the past and became its embodiment in the present. Any hat would fit him provided it was big enough. Seeing him in the robes of academic procession triggered *déja vu*'s of portraits of humanists seen in the great galleries. More than any, he was a Renaissance man, and the Elizabethan hat fitted him best of all.

Not always so. In earlier days before portliness set in, only the energy of the man, his bouncing step, the vivacious sparkle of his eye would have suggested such a picture. His springing from the balls of his feet while walking —and the same motion was evident when he stood up to talk or lecture—is said to have been a hangover of his days as a runner. He also played tennis and liked to swim. A six-month sabbatical leave in 1938 provided the opportunity to fly out on the China Clipper to the Philippines to obtain a rare form of primate. This adventure is described in a paper entitled "A Trip to Bohol in Quest of Tarsius." He included an entry from his diary telling of a stopover at Wake Island, part of which reads: "I had a field-day going in swimming, collecting shells and coral and all manner of lovely coelenterates. . . . The water was several degrees warmer than at Honolulu and the clearest water I have ever bathed in."

The quotation continued: "What a paradise Wake would be for anglers and zoologists, far more ideal than Bermuda for a biological station." Fishing to Fulton was a means of obtaining specimen! In his later years I found that if I ever forgot myself long enough to say I had been fishing or duck-shooting, he would look at me as much as to say, "Now what kind of a foreign language is this? Let's get on to another subject." For however much he may have enjoyed exercise when he was young, it was apparent that as he grew older he looked upon it as a glorious waste of time. A diabetic, such as he became, is helped by a little exercise. I was once foolish enough to suggest that he might enjoy riding a bicycle. He scowled, and that conveyed everything that St. Paul took several words to say: *When I became a man I put away childish things.*

Not much is known about Fulton as a child, not much, that is, that would satisfy a psychologist of today in trying to understand the mixture of forces that combined to give him the driving, energetic appearance of a young Lochinvar riding out of the west, with a wide-eyed look somewhat suggesting Nemesis in pursuit. The reverse of Peter Pan who never grew up, Fulton sometimes seemed never to have been a boy. This Citizen of the World came from the Middle West. It was easy to remember his age; he was born in St. Paul, Minnesota on 1 November just before the turn of the century. His mother's maiden name was Edith Stanley Wheaton. His father, John Farquhar Fulton, was an eminent ophthalmologist and was instrumental in establishing the University of Minnesota and its medical school.

In addition to parental influences and the affectionate esteem of four sisters, two school teachers must be credited with having done much to mould his early development. After giving up a "stamp company" begun at the age of 10 and "abandoned in bankruptcy" at 14, he began to collect stu-

dent editions of Marlowe, Rabelais, and Shakespeare under the influence of his German teacher, Charles Manckenburg in Northfield. At St. Paul's Central High School, his teacher, Miss Mary Jeanette Newson, took a great interest in guiding his reading, and she appears to have promoted his ambition to attend Harvard. It is said, however, that when working nights as a stack boy in the St. Paul Public Library, he spent so much time reading the great authors that he did not round out his preparation for the Harvard entrance examinations and failed.

At the age of 17 (1917) he entered the University of Minnesota. While listening to the lectures of Wallace Notestein* he developed a great interest in Milton, Boyle, and Digby, and subsequently began to collect their works. The Army interrupted his course of study at Minnesota, but he was to find compensation by being able as a "veteran" to enter Harvard without examination. As he liked to say, "The heavy artillery shot me into Harvard!" At Harvard his bibliophilic tendencies were further stimulated by Harold J. Laski and his zoology professor, Edward L. Mark, and his library grew to about 500 volumes. In 1921 he obtained his Bachelor of Science degree *magna cum laude,* and was to receive the same accolade with his Harvard medical degree in 1927.

The voice of the Swiss stewardess came over the loudspeaker in three languages to inform us that we were approaching London and to please fasten seat belts. Dr. Fulton's voice characteristically had the quality of one put through an amplifier. But like the stewardess' it was always pleasantly loud except when boisterously singing or thundering a Johnsonian "Sir" when he rapped for attention to voice an objection. So distinctive was his voice that one could not read his yearly printed Christmas letter without the sense of hearing him speak.

The sun which had been momentarily under a cloud came out again. . . . Gentleness, generosity; the refrain of oft-repeated kindnesses. His nervous system was more geared to the welfare of the species than to self-preservation. Almost never the sign of an ulterior motive; no wheedling to get things done. He thought and spoke in terms of "the Lab family." Gifts, things said, trickled out by way of the heart.

Yet outside enmity lingered near his door. Some found it insufferable to see the chauffeur waiting to drive him home to lunch and pointed to it as an indication of a lack of sensitivity on his part to the feelings of less affluent colleagues. Others begrudged him his large and able secretarial staff. Little winds were sown, and days came when the winds blew harder. Sometimes

* Now Professor Emeritus of English History at Yale.

they blew harsh things that were said right back to him. And when they began to blow some things away, one began to wonder how he could continue to be so charitable towards those who beset him. You would look at him in a way to ask as much, and he would answer with a characteristic wriggle of his eyebrows and a twinkle in his eye.

The landing gear whined into position, the wing flaps went down, and the plane took on a feeling of heaviness. In the last few years his face had assumed a heavy look, suggesting a little the ponderous look of Dr. Johnson in the Reynolds portrait. How would this reveal itself in the Sir Gerald Kelly portrait now on exhibition at the Royal Academy?

Fulton was a Fellow of the Royal College of Physicians and belonged to the Royal Society of Medicine. These and many other associations all bespoke his close identification with England and the large amount of time he spent there. It was natural to think of him in London at this time of the year. To those who knew his career it seemed natural, too, that Oxford had decided to honor him. Immediately after Harvard he had gone as a Rhodes Scholar to Magdalen College, Oxford, carrying with him a letter of introduction to Professor Charles Sherrington from Professor Walter B. Cannon. He was graduated D.Phil. in 1925. In this period he had conducted his study on muscular contraction and the reflex control of movement which was published as a book in 1926, after his admission to Harvard as a third-year medical student. In prefacing the bibliography of 1,066 references (a figure reminiscent of William the Conqueror!), he wrote: "In order to secure the greatest possible accuracy, each of the references in the following bibliography has been personally verified by the writer from the original sources after the titles were set up in galley proof." As Viets recently said of this book, "The orderly and readable account of a complex and little understood subject won high praise from both English and American scholars."

After a year as an Associate Neurological Fellow on the service of Dr. Harvey Cushing, he returned in 1928 for two more years at Oxford as a Fellow of Magdalen College and investigator in the laboratory of Charles Sherrington. Thirty years later at Liverpool, in beginning the Sherrington Lecture he said, "It is in the Hippocratic tradition to hold in reverence and affection those who have taught us the Art. For six years I had the great privilege of working under Sir Charles Sherrington's immediate guidance. . . ."

We were earthbound now in an airport bus and on the way to London. A schizophrenic young man (a former classics scholar and school teacher) sat down beside me and began to tell me about the ecstatic experiences he

had had in the plane that morning. Whitsuntide was coming, and on Sunday he would see the Holy Ghost! He proclaimed his delusional experiences with such conviction that it made one realize that a crazy man would be crazy not to believe in his crazy feelings.

Sherrington "disclaimed any credit for advancing knowledge of *mental functions.*" "What right," he asked, "have we to conjoin mental experience with physiological? No scientific right, only the right of what Keats, with that superior Shakespearean gift of his, dubbed 'busy common sense.'"* In the late 1920's, during the renaissance of the Yale Medical School, Dean Milton Winternitz was looking for just such a "busy common sense" when he heard of Dr. Fulton and prevailed upon him to take the Chair of Physiology. He came in 1930 and began to give *muscle* to the brain. In 1955 in the volume of this Journal celebrating his 25 years as Professor, it was said of him: "It is of consequence to research on mental disease, our country's foremost health problem, that long ago his interest in localization not only applied to the representation of bodily functions, but also transcended Sherringtonian tradition and extended to psychological processes. His use and promotion of interdisciplinary study for demonstrating correlations of brain function and psychological phenomena set the stage for lines of endeavor that promise to be fruitful for years to come."

Neurosurgical techniques (an innovation for physiology) were introduced into the new Yale Laboratory. So were monkeys and apes for neurophysiological research—an innovation for America. Almost from the beginning psychology walked hand in hand with physiology, and a few years later in 1935 at the International Congress in London, Fulton and Jacobsen were to report their notable findings on the effects of removing the tips of the frontal lobes in the chimpanzee. The remarkable emotional changes in the animal "Becky" suggested to Egaz Moniz the operation of frontal leukotomy to alleviate mental suffering. Although the operation no longer finds favor for this purpose, the clinical and experimental findings that grew out of it have greatly broadened knowledge of the function of the brain in intellectual and emotional behavior.

The Thirties proceeded. The period before the War. The Golden Decade in the Laboratory. Faces from far and wide. The seminar room lined with books—old books and new books—and new findings falling out of the pages of the old. New instrumentation. Electrophysiology developing. The steady production of books and papers. Dr. Fulton's universally read *Physiology of the Nervous System.*

* Quotations taken from J. F. F.'s appreciation of Sherrington. *(J. Neurophysiol.,* 952, *15,* 167-190) (italics mine).

Chelsea . . . Wellington Arch . . . Hyde Park.

In a few minutes I would be meeting Mrs. Fulton before proceeding to the hospital. A more familiar scene stole into the atmosphere of strangeness, and thoughts began ascending Deepwood Drive to the gracious, sprawling, Swiss-chalet house atop Mill Rock with its expansive view of New Haven Harbor. Gay feelings tugged against the sense of something parting: A summer evening on the screened-in porch . . . friends and laughter . . . the mid-day Sunday dinner . . . a centerpiece of flowers. . . .

Before my arrival there had been a remarkable improvement in Dr. Fulton's condition and he had regained consciousness. The outlook seemed favorable, and I prepared to return to Zürich. In the morning I went to the Middlesex Hospital to say "goodbye." He was still asleep, and I sat down in the corner of the darkened room to wait for him to waken. Soon the door opened and an old woman put in her head and shouted, "Want your *Times*, Sir?" He sat bolt upright in bed, reached for some change, and paid her. Before he realized I was in the room, he turned straight to the obituary page and began to search it intently. I stirred, and he looked my way and said, "By Jove!" and immediately launched into a pleasantry. The conversation strayed to the Continent, and he reminisced about the time that he had spent three months in Heidelberg "dissecting a leg." This led to a joke about a mutual acquaintance in Switzerland.

At the appointed time he went up to Oxford for the long anticipated ceremony. Not well enough to march in the academic procession, he waited in the theater. Then followed a painful flight home across the Atlantic and a prolonged convalescence in New London, New Hampshire. The three years that remained were perhaps to be more important than any in his development as an individual.

Man finds himself in the predicament that Nature had endowed him, essentially, with three brains which despite great differences in construction must function together. The oldest of these brains is basically reptilian. The second has been inherited from the lower mammals, and the third is a late mammalian development which has made man peculiarly man. Investigations of the last 20 years, in which Dr. Fulton's Laboratory played a prominent role, have demonstrated that the lower mammalian brain, or so-called limbic system, which we share with all mammals, functions at a primitive level in emotional behavior. It is inferred that it elaborates basic emotional and visceral feelings that guide behavior essential for self-preservation and the preservation of the species. Hence it has been referred to as "the visceral brain," with visceral being used in its 16th century meaning and connoting strong inward feelings.

The problem of communication existing between man's intellectual brain and his "visceral brain" has been metaphorically compared to that of a rider and his horse. Many men knew Dr. Fulton the man, but it is doubtful that any, least of all himself, knew or understood his horse. However enlightened he may have been as a physiologist, his veterinary medicine was of an Elizabethan vintage, and his prescriptions were Oslerian. His bedside table looked like an apothecary's shop. Possibly it was because his horse was more spirited and bounding with energy than most; whatever the reason, in later years his friends looked apprehensively on, wondering if he would ever break his horse, or whether it would be the breaking of him.

The next time I saw him, 14 months after London, there was no question of who was in control. The place now was London, Ontario; the occasion, a board meeting of the *Journal of Neurophysiology* which he had called during the autumn meetings of the American Physiological Society. Some 50 pounds lighter, he was reminiscent of the lean and handsome man who had taught me physiology. The voice had lost its resounding quality, but it was the voice of assurance.

His splendid recovery continued. Sprightly letters were written. One arrived in January of this year saying he would be in Washington on February 4th. This was the last time I saw him. He came to the National Institutes of Health in Bethesda as a member of a committee which reviewed applications for support of the history of medicine. Ever since Oxford days when he had been welcomed into "The Open Arms" by Lady Osler and had been inspired by Osler's library, he had been a contributor to the history of medicine. *Selected Readings in the History of Physiology,* which appeared in 1930, was followed by a long succession of books and papers, including his well-known bibliography of Robert Boyle and the extensive life of Harvey Cushing. His own library, given to Yale, contained more than 10,000 items and was resplendent with special collections of Boyle, Digby, Priestley, Willis, Whytt, and others. Royalties from his books and other writings, honoraria, and most of his salary as Sterling Professor were donated to augment this original gift.

In the evening he came to dinner at our house in the country near the town of Potomac. His close and longtime friends the Robert Livingstons joined us. He was full of lively talk about the new developments at Yale and during dinner told us about the fulfillment of a program for which he had been working ever since assuming the new chair of the history of medicine in 1951. Step by step he related the recent developments that led to administrative approval of a combined Department of the History of Science and Medicine and the enthusiastic endorsement of Derek J. de Solla Price

for the newly created professorship of the history of science. For the first time the University would offer graduate degrees in the history of science and medicine.

It came time for him to go, and I prepared to drive him to his hotel. We walked out to the car. The night air carried familiar smells of the farm. The tinkle of Swiss bells on the goats and sheep made music in the meadow. Driving in, we talked of old times, old friends, the Folger Library. He mentioned stoically that enlarging cataracts were making it difficult for him to read. The doorman greeted us. Inside the hotel entrance we said "goodbye." He turned and walked slowly away. My eyes lingered. In his footsteps there seemed to be an echo of Milton's *Samson Agonites*—

And calm of mind, all passion spent.

WILLIAM A. TISDALE*
GERALD KLATSKIN**

Department of Internal Medicine, Yale University School of Medicine

THE FEVER OF LAENNEC'S CIRRHOSIS†

Fever is not regarded by most physicians as a prominent or common clinical manifestation of uncomplicated Laennec's cirrhosis. When present, it is generally attributed to intercurrent infection, gastrointestinal hemorrhage or, less commonly, to complicating hepatoma. However, there is reason to believe that the cirrhotic process itself may give rise to fever.

Although earlier observers had commented on the fever seen occasionally in alcoholic patients with cirrhosis,[19, 24] Carrington[8] first documented its frequency (41 per cent) in uncomplicated cases. Almost a quarter of Eppinger's cases had fever apparently related to cirrhosis itself.[10] Other reports[1, 11, 13, 15, 27] have mentioned fever as a clinical sign of cirrhosis, but few of these have included sufficient clinical, bacteriological, and pathological details to permit accurate conclusions about the significance of pyrexia in this disease.

In studying a large group of alcoholic patients with Laennec's cirrhosis, we have been impressed by the frequent occurrence of fever, often prolonged in duration and striking in degree. In some patients, the pyrexia has been clearly related to associated infection or neoplasm, but in many no cause other than the liver disease itself has been demonstrated. Often the fever has been a matter of great concern and has led to fruitless diagnostic procedures and to ineffective therapeutic trials of antibiotics.

We have reviewed our experience with patients having proven Laennec's cirrhosis in order to determine the incidence, characteristics, and significance of fever in this disease.

MATERIALS AND METHODS

The 150 patients included in this study were drawn from a group of over 300 individuals with histologically established Laennec's cirrhosis admitted to the Grace-New Haven Community Hospital in the years 1948-59. Completeness of medical

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** Professor of Medicine.

† Supported by a research grant (A-714) from the National Institute of Arthritis and Metabolic Diseases National Institutes of Health, U.S. Public Health Service.

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evaluation was the sole basis for selection of this study group. Practically all of the patients admitted to longstanding, heavy alcohol consumption and poor dietary habits, and the physical examination often revealed signs of chronic liver disease and its complications. In addition to routine blood and urine examination, all patients had one or more sets of liver function tests[19] shortly after admission. In all cases with fever, appropriate bacteriological cultures of body fluids and secretions were obtained, and roentgenograms of the chest were available for all patients. Definite Laennec's cirrhosis was demonstrated in all cases on tissue obtained by percutaneous Vim-Silverman needle biopsy of the liver. Operative and necropsy specimens were not included in this study to avoid any morphological artefacts resulting from surgical trauma or post-mortem autolysis. Most of the biopsies were performed early in the patient's

TABLE 1. FEVER IN LAENNEC'S CIRRHOSIS

	Number	*Per cent*
1. Fever with infection	22	14.7
2. Fever with no apparent infection	58	38.6
3. No fever	70	46.7
TOTAL	150	100.0

hospital stay, and, in the cases with fever the biopsies were carried out during febrile periods. Patients were excluded from this study because of inadequate clinical, laboratory, bacteriological, or pathological information and, in many instances, because of brief hospitalization that prevented proper evaluation of their temperature patterns.

Patients were considered to have significant fever if their rectal temperatures exceeded 100.5° F. on three or more consecutive hospital days.

RESULTS

Incidence of fever in Laennec's cirrhosis. As indicated in Table 1, 80 of the 150 patients with cirrhosis had fever. Of these 22 (15 per cent of the entire series) had well-documented infections to account for the fever, while 58 (39 per cent of the entire series) had only active cirrhosis as a cause of fever. In contrast, only 3 patients (12 per cent) in a group of 25 cases with proven postnecrotic cirrhosis had significant fever during hospitalization.[21]

Characteristics of the fever in uncomplicated Laennec's cirrhosis. About 25 per cent of the 58 patients with the fever of uncomplicated cirrhosis had low-grade temperature elevations of less than 101° F., and a few (7 per cent) had striking fevers of over 103° F. (Table 2). Most of this febrile group, however, had fever of a moderate degree, with peaks ranging from 101 to 103° F. The figures summarizing the duration of fever are not strictly accurate, for a few patients left the hospital before their tempera-

tures reached normal levels. Nonetheless, over half the cases had fever that persisted for more than two weeks and almost a quarter (22 per cent) had daily temperatures of over 100.5° F. for longer than four weeks. In most instances the fever decreased gradually and, once absent, recurred only with the onset of complicating gastrointestinal hemorrhage or infection. As noted in Table 2, shaking chills were distinctly uncommon, and no single pattern of fever seemed characteristic of these cases.

TABLE 2. CHARACTERISTICS OF FEVER IN 58 PATIENTS WITHOUT APPARENT INFECTION

	Number	*Per cent*
1. Maximal height (°F. p.r.)		
< 101	15	26
101-103	39	67
> 103	4	7
2. Duration		
< 1 week	10	17
1-2 weeks	14	24
2-4 weeks	21	37
4-8 weeks	10	17
> 8 weeks	3	5
3. Chills	2	3
4. Type		
intermittent	31	53
remittent	26	45
hectic	1	2

Clinical and laboratory features of patients with the fever of uncomplicated cirrhosis. Clinically, the 58 febrile cirrhotic patients differed in certain important respects from the 70 afebrile patients (Figs. 1 and 2). While the afebrile group showed the usual male sex predominance (66 per cent), female patients accounted for about 60 per cent of the febrile group. Even more impressive was the observation that over two-thirds (68 per cent) of the febrile women cirrhotics were less than 50 years of age, many being in their late twenties or early thirties. In contrast, only 30 per cent of the afebrile women and 42 per cent of the febrile males were under 50 years of age. The patients with fever drank more heavily and had correspondingly poorer diets. Both the acute and late mortality figures in the two patient

groups were surprisingly low, a result, in part, of the difficulty in obtaining careful follow-up studies in alcoholics.

The patients with fever had more impressive physical signs of illness and active hepatic disease than did those without fever (Fig. 2). Half of the febrile cirrhotics were severely malnourished, practically all were jaundiced, and many had abundant ancillary evidence of advanced liver disease. Palpable hepatomegaly was found more common in the group of febrile individ-

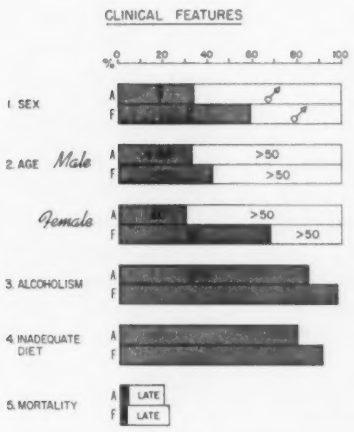

FIG. 1. Comparison of certain clinical features in afebrile (A) and febrile (F) patients with Laennec's cirrhosis.

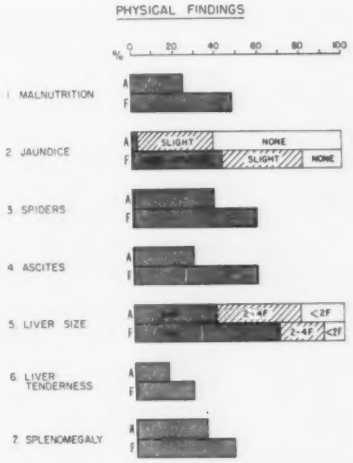

FIG. 2. Comparison of physical findings in afebrile (A) and febrile (F) patients with Laennec's cirrhosis.

uals, and definite hepatic tenderness was elicited in about a quarter of these patients. Splenomegaly was common in both groups.

Routine laboratory examinations and liver function tests confirmed the clinical impression that the cirrhotics with fever had more severe liver disease than did those without fever (Fig. 3). About 20 per cent of the febrile group had marked anemia, related in part to gastrointestinal hemorrhage. Two-thirds of the febrile cases had a leukocytosis in excess of 10,000 per mm^3., one-third having more than 15,000 leukocytes per mm^3. Most of those with fever had hyperbilirubinemia and marked bromsulphalein retention, though the interpretation of this latter observation was rendered uncertain by the fever and deep jaundice. Almost 60 per cent of the febrile cirrhotics had elevated serum levels of alkaline phosphatase, 10 per cent having levels

of more than twice normal by the method used, while only 28 per cent of the 70 febrile patients had abnormal values. Significant hypoalbuminemia and hyperglobulinemia were found with about equal frequency in the two groups.

Pathological features of Laennec's cirrhosis with fever. In analyzing the histological features of the biopsy specimens obtained in febrile and afebrile patients, special attention was directed to the type and extent of parenchymal cell degeneration, necrosis and regeneration, the degree of connective

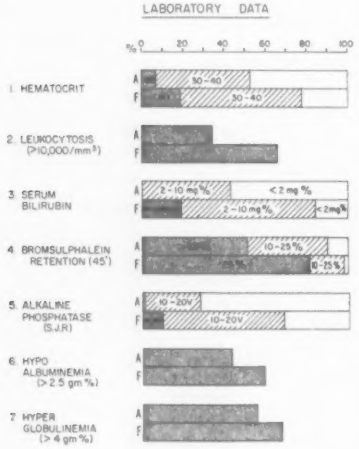

FIG. 3. Comparison of certain laboratory findings in afebrile (A) and febrile (F) patients with Laennec's cirrhosis.

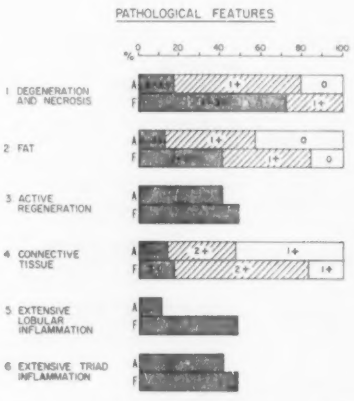

FIG. 4. Comparison of pathological features in afebrile (A) and febrile (F) patients with Laennec's cirrhosis.

tissue collapse and thickening, and the nature and intensity of lobular and portal inflammation.

Figure 4 compares the findings in the afebrile group with those in the febrile group without infectious complications. Most impressive was the advanced degree of hepatocellular damage seen in the latter. Almost three-quarters of the specimens showed extensive disruption of lobular architecture, marked variation in liver cell size, nuclear swelling or pyknosis, and the presence of abundant cytoplasmic hyalin inclusions or so-called Mallory bodies. In the remaining cases, these changes were present also but were less marked and more patchy in distribution. In contrast, only 17 per cent of the 70 afebrile patients had advanced hepatocellular changes, a majority demonstrating either no active parenchymal destruction or minimal focal changes.

Fatty vacuolization of liver cells was marked in about 40 per cent of the febrile cases and was slight in extent in an equal number. Only rarely (13 per cent) was extensive fatty infiltration noted in the afebrile patients, most of them showing only slight (44 per cent) or no (43 per cent) infiltration.

Regenerative activity in the parenchyma, as manifested by mitotic figures, double nuclei, and thickening of the liver cell plates, was equally common in both groups.

Connective tissue was more abundant in the biopsy specimens of the febrile patients, usually due to the presence of zones of thickened and collapsed reticulum at sites of recent liver cell loss in addition to the usual deposits of collagen.

Parenchymal infiltration by polymorphonuclear leukocytes in the zones of active necrosis was a notable feature in 48 per cent of the febrile cases, this process paralleling closely the extent of liver cell destruction and Mallory body formation. As described by Mallory,[21] the inflammatory cells surrounded and, in some areas, invaded the dying liver cells. Only 11 per cent of the afebrile group had comparable parenchymal inflammatory changes.

In contrast, neither the extent nor predominant cell type of the portal tract inflammatory response was distinctive in the cases with fever, about half of both groups having moderate to heavy infiltration with both polymorphonuclear and mononuclear leukocytes.

Repeat needle biopsies were performed on 9 of the 58 febrile cirrhotic patients following the subsidence of fever, at intervals varying from 18 days to 3½ years after the initial biopsy. Microscopically, the most consistent (7 of 9 cases) and impressive differences between the two specimens were the subsidence of parenchymal necrosis and the decrease in intralobular inflammation.

Thus, while there were no distinctive morphologic differences between the febrile and afebrile cases, both liver cell damage and intralobular inflammation were more striking in those with fever.

Febrile conditions complicating Laennec's cirrhosis. As noted previously, 22, or 15 per cent, of the 150 patients had fever related to infections or other inflammatory diseases complicating cirrhosis. In each case, the secondary diagnosis was established by bacteriological and/or other studies, and, in many instances, it was confirmed by the rapid clinical response to appropriate antibiotic therapy.

The sites and types of infection encountered in these 22 patients are summarized in Table 3. In 10 cases the urinary tract was infected by Gram-negative organisms. Pulmonary sepsis, usually caused by Gram-positive

cocci, was the second most frequent complication (8 cases), while tuberculosis, long considered a special hazard of cirrhotic patients, was present in only 3 instances. Acute pancreatitis, another illness frequently associated with alcoholism and Laennec's cirrhosis, was encountered only once in this series.

TABLE 3. NATURE OF INFECTIONS IN 150 PATIENTS WITH LAENNEC'S CIRRHOSIS

1. Patients with proven infection	22
2. Type of infection	
a) *Genitourinary* (cystitis, pyelonephritis) E. coli—6, proteus—2, aerogenes—1, pseudomonas—1 (2 episodes of bacteremia)	10
b) *Pneumonitis* pneumococcus—4, staphylococcus—2, unknown—2	8
c) *Tuberculosis* pulmonary—2, miliary—1	3
d) *Meningitis* unknown	1
e) *Abdominal abscess* unknown	1
f) *Pancreatitis* unknown	1

Acute bacteremia was demonstrated in 6 of these 22 febrile patients (Table 4) and was due to the colon bacillus in 4 instances. While the bloodstream seeding was secondary to a urinary tract infection in 2 of these latter patients, no primary tissue focus was demonstrated in the other 2. Recently, we have encountered 2 other cirrhotic patients, not included in this study, with "primary" *E. coli* bacteremia.

In Table 5, certain clinical and pathological features of the 22 cases with fever and proven infection are contrasted with those of the 58 febrile cases without demonstrable infection. The unusual sex distribution previously described for the uncomplicated febrile cirrhotics was absent in the patients with infection: in the latter group, males predominated and only 30 per cent of the women were under 50 years of age. The fever that accompanied infection was often high and of short duration, typically resolving rapidly with the administration of proper antibiotic therapy. Shaking chills were noted by at least a quarter of the patients with infection.

TABLE 4. BACTEREMIA IN 150 PATIENTS WITH LAENNEC'S CIRRHOSIS

Organism	*Cases*	*Local infection*
E. coli	4	Genitourinary—2 None (PM)—1 (clinical)—1
Pneumococcus	1	Lobar pneumonia
Staphylococcus aureus	1	Pancreatitis

TABLE 5. COMPARISON OF CLINICAL AND PATHOLOGICAL FEATURES OF FEBRILE CIRRHOTIC PATIENTS WITH AND WITHOUT INFECTION

	Febrile with infection (22 Cases) %	*Febrile without infection (68 Cases) %*
CLINICAL		
Sex—female	45	59
Age— < 50 yrs. Male	17	42
Female	30	68
Fever— > 102° F.	68	31
Fever— > 2 wks.	18	59
Chills	27	3
Mortality, acute	14	4
PHYSICAL SIGNS		
Jaundice— > mod.	23	43
Hepatomegaly— > 4F	64	69
Splenomegaly	14	47
Ascties	41	59
LABORATORY DATA		
Leukocytosis, > 10,000/mm.^{3}	77	66
Bilirubin, total > 10 mg.%	9	19
Alkaline phosphatase, > 10 u. S.J.R.	24	59
PATHOLOGICAL FEATURES		
Liver cell necrosis, > mod.	65	72
Fat, > mod.	35	41
Lobular inflammation, > mod.	60	48

The superimposed infectious conditions added somewhat to the acute mortality of this group, but only 2 patients died as a direct consequence of the infection. From a clinical standpoint, the individuals with infections resembled those *without* fever, in that their disease appeared less severe than those with cirrhotic fever. However, histologically their livers showed the extensive liver cell damage and lobular inflammation seen in the patients with fever due to cirrhosis.

COMMENT

The occurrence of fever unrelated to infection or neoplasm in 39 per cent of 150 patients with Laennec's cirrhosis illustrates the frequency with which pyrexia occurs in this disease. It is noteworthy that, in contrast, only 12 per cent of 25 patients with uncomplicated postnecrotic cirrhosis had fever.

While the fever in this group was moderate in degree, the maximum rectal temperatures ranging between 101-103° F., it was characteristically prolonged and persistent. Gradual subsidence of the fever began usually during the third or fourth week of hospitalization as general clinical improvement became apparent. Recurrence of fever was uncommon and usually indicated intercurrent infection or gastrointestinal bleeding. True shaking chills and hectic fever patterns were rare and there was no characteristic fever pattern. In general these observations confirm those of others,[1, 8, 10, 11, 18, 27] cirrhotic fever having been described as low-grade, protracted, and either remittent or intermittent in type. Finally, as noted previously by Levrat *et al,*[20] antibiotic therapy had no effect on the pattern or duration of fever in any case.

Although the pyrexia of cirrhosis appeared in both sexes and at all adult age levels, women under 50 years of age seemed uniquely prone to manifest fever. We were unable to correlate this observation with exorbitantly high alcoholic consumption or obvious endocrine dysfunction on the part of these female patients. Others[25] have commented on the relatively early occurrence of alcoholic cirrhosis, its complications, and frequently fatal outcome in females. This tendency of young women to react dramatically to chronic active liver disease is not unique to Laennec's cirrhosis and is also evident in the case of women having postnecrotic cirrhosis.[3]

Significant fever was distinctly uncommon in individuals with no overt clinical manifestations of active liver disease, such as jaundice, hepatomegaly, and fluid retention, but, conversely, signs of flagrant hepatic disease were not necessarily associated with fever. Unlike the patients with alcoholic cirrhosis described by Summerskill *et al.*[26] jaundice was not invariably accompanied by fever in our experience. The leukopenia often reported in

Laennec's cirrhosis was rare in both the febrile and afebrile patients, the total leukocyte count being less than 5,000 per mm^3. in only 3.5 and 4.3 per cent of the groups, respectively. Leukocytosis was the rule in febrile cases, counts greater than 15,000 per mm^3. occurring in about a third of these patients, a finding consistent with many previous studies.[1, 11, 13, 18] This leukocytosis usually diminished as fever lysed, suggesting a common inflammatory stimulus.

As with the clinical manifestations, fever occurred only rarely in the absence of grossly deranged liver function tests, but extensive abnormalities in liver function were not necessarily accompanied by fever. Neither the degree nor duration of pyrexia correlated well with any single functional disturbance. Of interest was the frequency of elevated levels of serum alkaline phosphatase, since many determinations fell into the range commonly associated with bile duct obstruction.[14] These, along with fever and leukocytosis, often suggested the possibility of biliary tract disease or neoplastic involvement of the liver.

No truly specific morphological picture characterized the livers of the cirrhotic patients with fever. However, in addition to the other changes typical of Laennec's cirrhosis, evidence of active and often extensive liver cell damage was present in all the febrile cases. In contrast, a fifth of the afebrile group had no demonstrable cell necrosis and only one of the 70 biopsy specimens showed the extreme degree of active parenchymal destruction present in 30 per cent of the febrile group. Significant intralobular inflammation, presumably a response to the local tissue necrosis,[26] also seemed typical of the cirrhotic process in those with fever. Finally, the rough parallelism between the subsidence of fever and loss of parenchymal necrosis and inflammation in the 9 individuals studied serially suggested a definite relationship between clinical fever and these morphologic changes. Phillips and Davidson,[25] in a careful clinico-pathological study of acute liver failure in alcoholics, found these same cellular and inflammatory features to be characteristic of patients with severe, often fatal, cirrhosis. In addition, many of their subjects were young and had fever and leukocytosis as prominent clinical signs. These findings suggest that liver cell necrosis and reactive inflammation are related, perhaps etiologically, to clinical fever. From a practical standpoint, fever should not be attributed solely to Laennec's cirrhosis if liver biopsy fails to demonstrate active parenchymal necrosis and inflammation.

The frequent occurrence of pyrexia in patients with histologically active but uncomplicated cirrhosis suggested that specific disorders of body temperature regulation may be characteristic of this disease. Decreased sweat-

ing and abnormal collections of extracellular fluid, two factors considered capable of elevating body temperature,[4, 20] were not present in all the febrile cases and cannot be accepted as major causes of fever in this group. Cryptic bacterial infections, undetected by the methods used in this study, might stimulate fever production, especially in view of Heyman and Beeson's demonstration that cirrhotics are unduly sensitive to administered bacterial pyrogens.[16] Against this theory is the failure of 20 courses of antibiotic therapy, including 5 of orally administered neomycin, to affect the fever of 17 patients. Similar results and conclusions were reported by Levrat *et al.*[30] Pyrogenic substances released from necrotic liver cells or polymorphonuclear leukocytes[5] were considered a more likely factor in the pathogenesis of fever in view of the striking association between pyrexia and such morphological alterations. However, the occasional lack of parallelism between these two features indicated that other mechanisms were involved at least in part. Finally, since certain pregnane steroid compounds from adrenal and gonadal sources have been shown to be pyrogenic in man,[6, 17] the possibility was considered that the fever of cirrhosis might stem from the action of incompletely conjugated and unexcreted compounds normally inactivated by the liver. To test this hypothesis, Dr. George L. Cohn of the Yale Department of Internal Medicine analyzed the plasma of 16 cirrhotic patients, 6 of whom were febrile, for unconjugated etiocholanolone. Preliminary results[9] indicate that the plasma level of free etiocholanolone is raised in some cases but is not correlated with the presence of fever. Further studies are needed before any valid conclusions can be drawn regarding the possible role of disordered steroid metabolism in the pathogenesis of fever in cirrhosis.

The 15 per cent over-all incidence of complicating infections and other inflammatory conditions in these 150 patients with Laennec's cirrhosis is lower than that recorded by many other authors,[27] especially by those reviewing autopsied cases.[15, 28] Except for the rarity of tuberculosis (and notably the absence of tuberculous peritonitis) in our series, the types of infections resemble those described by others, genitourinary and pulmonary sepsis being the most frequent. The clinical response to antibiotic therapy and the low mortality from the infections themselves indicate that cirrhotic patients tolerate intercurrent infections quite well. Only the unduly high incidence of coliform bacteremias seems unique in these individuals, our experience confirming that of Whipple and Harris,[28] Martin *et al.*,[23] and Caroli and Platteborse.[7] The specific alterations in host resistance that permit such bloodstream invasion by *E. coli* organisms, often without obvious primary septic foci, are not yet known. Abnormal growth of organisms in the gas-

trointestinal tract,[28] lowered local resistance to bacterial invasion of the bowel wall, bypassing of the hepatic circulation by collateral venous channels,[28] and impaired humoral mechanisms of bacterial killing[3] all may contribute to this association of cirrhosis and coliform bacteremia.

In terms of their liver disease, the 22 patients with intercurrent infection were not particularly ill and had shorter hospitalizations than did those with the fever due to cirrhosis itself. Short-lived high and hectic fever, frequent shaking chills, and prompt subsidence of fever by crisis after the institution of antibiotic therapy are the clinical features that characterized the course of these patients. The clinical picture and the pathological findings in the cirrhotics with infection suggest that acute sepsis may activate the processes of liver cell necrosis and reactive inflammation, but that prompt and specific antibacterial therapy permits recovery in most instances.

SUMMARY

In a clinico-pathological study of 150 patients with Laennec's cirrhosis, fever was present in 80 cases. Active and uncomplicated cirrhosis seemed the sole cause of fever in 58 instances, intercurrent inflammatory conditions accounting for the other 22 febrile cases. The fever due to cirrhosis was typically moderate in degree, prolonged in duration, and occurred in patients with clinical, laboratory, and pathological evidence of active and advanced hepatic disease. The pathogenesis of the fever of cirrhosis remains uncertain but may be related in part to hepatic necrosis and inflammation and to disturbances in the metabolism of certain steroid hormones.

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ASEXUAL REPRODUCTION IN ACOELOUS TURBELLARIA†

Among the turbellarian flatworms reproduction is typically associated with a sexual process. However, in certain orders such as the Tricladida and Rhabdocoela asexual reproduction is also recognized.[a] The purpose of this short paper is to report the discovery of asexual reproduction by what is probably a fission-like process in another order of Turbellaria, the Acoela.

Asexual reproduction was discovered in immature forms of an acoel worm identifiable as *Amphiscolops langerhansi* (Graff) except for the fact that it lacked the statocyst typically present in this species. However, absence of the statocyst can be explained as a consequence of asexual reproduction. This will be made clear after the data in support of asexual reproduction have been presented and these data comprise both indirect evidence as well as direct evidence.

During the course of culturing immature *A. langerhansi* (worms 2-3 mm. in length, no visible sexual organs) there appeared in the cultures, quite consistently, small odd-shaped worms (Fig. 1). These forms, though markedly different from the larger worms in the culture relative to size and external morphology, did seem to be some form of *A. langerhansi*: first, because their pigmentation (concrement granules and symbiotic algae) and bilobed posteriors closely resembled the same characters in the larger worms; second, because these were pure cultures and there was no possible source of other flatworms. Such forms were occasionally seen in the aquaria, but were hard to find because of their small size.

To determine whether or not these small worms appeared in cultures containing only one of the larger worms, five of the immature worms were isolated into separate culture depressions. Observations were made daily for varying lengths of time and the new, odd-shaped worms removed as they appeared. The overall data are as follows: Odd-shaped little worms appeared in each of the culture depressions containing a single immature worm. These worms appeared one at a time, that is—within any period of

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observation only one new worm was discovered in a culture. In the first two days a new worm was discovered every 24 hours in the five cultures, but later this rate slowed down, the maximum for one culture being nine new worms in two and a half weeks. The new worms always appeared during the absence of light for they were usually discovered in the morning although in a few cases they appeared around midnight and in one case one appeared an hour or so after sunset. There were never any eggs observed in these cultures and in two cases where observations were made a few hours apart with a new worm appearing in the interval between observations, examinations of the larger worm gave the definite impression of a certain amount of truncation. The new worm was of such dimensions that it appeared as if it could fit perfectly its anterior margin to the posterior margin of the larger worm. These new forms do not resemble newly hatched sexual progeny (Fig. 2): for they are pigmented whereas the sexual progeny are colorless. Moreover, they lack statocysts where the sexual progeny do not and they are broader than they are long, which is the reverse of the sexual progeny. These odd-shaped worms remain quiescent for a day or so and then become more active as their body shape changes, becoming narrower and longer. This occurs first anteriorly, which happens to be the most active part of the body but eventually the whole worm is transformed into a small (0.5 to 1.0 mm. long), slim *A. langerhansi*. In two instances, after isolation and about two weeks growth, these slim worms were found to have present in their culture depressions, little odd-shaped worms similar to those from which they were originally derived.

The obvious conclusion from these data is that the immature *A. langerhansi* are capable of asexual reproduction. The major points favoring this interpretation are: i) The sexual immaturity of the isolated worms, ii) The absence of eggs, iii) The differences between the odd-shaped worms and progeny of known sexual origin which exclude the possibility that the odd-shaped worms are any sort of atypical sexual progeny, iv) The appearance of new forms one at a time and from a single parent, as would be expected of a fission-like process, v) The highly suggestive correspondence in the morphology of the anterior margin of the new forms with the posterior margin of the larger worms.

An alternative explanation might be that culture conditions were not normal and that the new forms represent at best an aberrant sort of asexual reproduction—a response to stress conditions. This is thought to be unlikely since normal appearing *A. langerhansi* developed from these odd-shaped forms. Also a few of these odd-shaped forms were found in the aquaria where living conditions are most probably quite normal. To us the

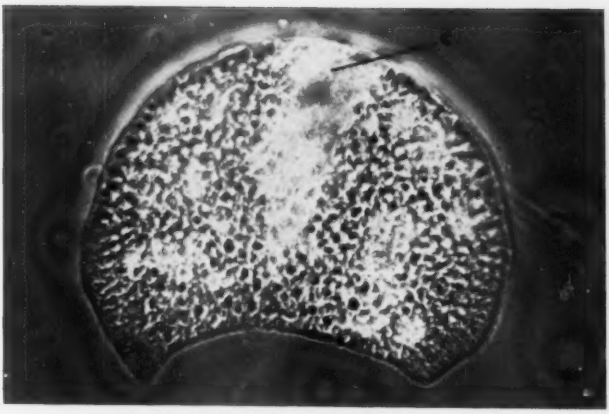


FIG. 1. Dorsal view of a whole mount of the asexual progeny. Anterior end at point of most refractive material or concrement (c). Fixed (4 parts formalin: 3 parts USP 95 per cent ethyl alcohol: 1 part glacial acetic acid); unstained; phase contrast.

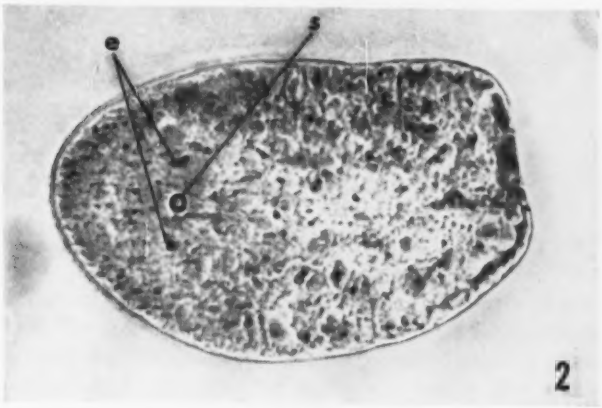
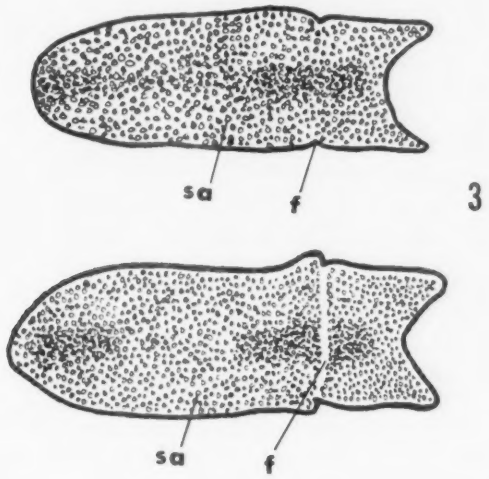


FIG. 2. Newly hatched sexual progeny. Live, showing eyespots (e) and statocyst (s). Phase contrast.

evidence is sufficiently clear to justify the conclusion that asexual reproduction does occur in *A. langerhansi.*

Attempts were made to observe the actual process of asexual reproduction, that is, to obtain direct evidence. It is probable that the actual asexual process can only be observed under special conditions, of which the key one is thought to be the virtual omission of daylight or light produced by fluores-



FIGS. 3 and 4. Drawings of immature worms showing symbiotic algae (sa) and lateral indentations inferred to be the incipient fission plane (f).

cent or filament-type illuminators. The basis for this general statement is three-fold. First, it will be recalled that all the asexual reproduction thus far described took place after daylight had passed; in fact no asexual progeny were known to appear in the presence of visible light. Second, preliminary work indicated that if worms known to be able to reproduce asexually were kept under constant illumination for 24 or 48 hour periods and then placed in the dark, asexual progeny, if they were going to be produced, appeared within an hour or two. Third, in two cases where the asexual process seemed to be in progress, the process was stopped at that point when the organisms were illuminated for purposes of observation.

Turning to the two cases of presumed direct observation, the data are these. In the first case an immature *A. langerhansi* was seen with lateral

indentations (Fig. 3) at the position expected from indirect inferences to be the site of cutting off of the asexual offspring. Three hours of subsequent intermittent observation showed no change from the initial condition. In this case observations were made at night. In the second case an animal was observed which had been kept under constant illumination for 24 hours and then transferred to a small cardboard box. This box was probably light-tight and had apertures, fitted with covers, above and below the culture containing the worm. This assembly was placed on the stage of a dissecting microscope and at intervals of ten to fifteen minutes the apertures were opened, allowing light to be transmitted through the culture, and observations of five to ten seconds' duration were made. Using this technique, only one worm was seen in apparent fission but this was because of lack of time, the technique having been evolved just a few days before the terminus of the project. The author is convinced that this approach or some modification of it, is the method of choice for the study of this process. In any case, what was observed after an hour and a half in the box is shown in Figure 4. The lateral indentations were joined by a narrow, colorless transverse stripe, an area devoid of algae, which is interpretable as the fission plane. A half-hour later with the animal unchanged, it was made into a whole mount. The pressure from the flattening, which accompanied the fixing, practically obliterated the clear area by forcing algae to fill it, hence, the slide is not as striking as it might be.

In all probability, the interest of this preliminary report on the actual asexual process rests more in the nature of the problems surrounding its elucidation, in particular the postulated inhibitory effect of light, than it does on the actual occurrence of the process. However, to the author's knowledge, this is the first reported case of a true fission-like mode of asexual reproduction in the Acoela. "Architomy" has been reported in an undescribed species of *Convoluta*,[5] the term Architomy referring to asexual reproduction by fragmentation into two or more viable parts by mechanical forces external to the organism, without preparation for such fragmentation by the organism. This is not what is commonly termed fission, for in this process, the fission plane is usually fairly well-defined and the forces separating the fission products are not external to the organism, but generated within it. Visible developmental changes may or may not precede the appearance of the fission plane. In *A. langerhansi*, although the observations are incomplete, there seems to be very little change in morphology preceding fission. The fission plane is fairly precisely definable, and the separation of the fission products is probably achieved by forces developed by the organism. This case is somewhat like the asexual reproduction of certain fresh-water planarians.[8]

There still remains one point to be discussed, the designation of these statocystless forms as *A. langerhansi,* for, as mentioned earlier, *A. langerhansi* typically does have a statocyst. The major reason for this designation is that the description of mature *A. langerhansi*[1,2] fits the mature statocystless worms in all anatomical details examined except, of course, for absence of the statocyst. Furthermore, the copulatory behavior and egg-laying of mature statocystless forms and the subsequent hatching of young which did possess statocysts (Fig. 2), is also like that reported for *A. langerhansi.* Is there, now, any explanation for the absence of the statocyst in these forms? Asexual reproduction supplies such an explanation for when acoels are cut in two transversely, the posterior portion reforms the missing anterior end but does not form a so-called ganglion or a statocyst.[4] In many respects the asexual reproduction we have just reported is like the regenerating posterior end of an acoel; if such a regenerating portion of an acoel cannot form a statocyst then, reasonably, an asexual offspring from the posterior end could probably not do so either. If we assume now that the population arose from a few immature worms giving rise to asexual progeny, which in turn produced more such offspring, we can understand how a large number of statocystless worms could arise. Furthermore since the worms were first discovered about mid-July and their numbers decreased sharply about mid-August, it is possible that the sexual progeny carrying statocysts never had time to become established as part of the aquarium population.

SUMMARY

Immature acoel worms, lacking a statocyst, but otherwise identifiable as *Amphiscolops langerhansi,* produce progeny under conditions which indicate that these worms are capable of asexual reproduction. Attempts to observe the actual asexual process have been partially successful despite the fact that visible light seems to inhibit the process. The presence of asexual reproduction can explain the absence of statocysts in these forms.

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THE PRECIPITATION OF SERUM LIPOPROTEINS BY MUCOPOLYSACCHARIDES EXTRACTED FROM AORTIC TISSUE†

Because of the frequent association of metachromatic staining material and lipid deposits within the artery wall, the suggestion has been made that the mucopolysaccharides of the connective tissue of arteries may in some way render lipids of the blood insoluble and thereby set the stage for the development of the lesions of arteriosclerosis.[1, 23, 28] Studies in this laboratory have demonstrated the accumulation of intravenously injected lipoproteins or of chylomicrons in regions of focal arterial injury, where metachromatic materials are present.[29, 30] Analyses of sulfate content in human aortas suggest that sulfated polysaccharides may cause deposition of the lipids by some chemical interaction.[28]

It is known that hyaluronic acid precipitates protein from diluted, acidified horse serum.[19, 24] Concentrated solutions of hyaluronic acid precipitate some protein from human plasma below pH 7.0.[20] Both hyaluronic acid and chondroitin sulfuric acid precipitate globin at neutral pH.[17, 18] A number of recent studies have demonstrated a rather specific precipitation of plasma lipoproteins by sulfated polysaccharides; these include sulfated dextran,[29] heparin,[7] agar agar,[5] and sulfated amylopectin.[8] Dyrbye and Kirk[10, 11] have extracted various mucopolysaccharides from aortic tissue, identifying a relatively high percentage of sulfated polysaccharides.

The *in vitro* precipitation of plasma lipoproteins by polysaccharides of the artery wall, to our knowledge, has not been demonstrated. An investigation of possible interactions between these substances was begun as part of a continuing investigation in this laboratory of the pathogenesis of arteriosclerosis.

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METHODS

Large sections of 30 human aortas, all from adults over the age of 25, were collected from the autopsy service and pooled. These were frozen immediately. Later the intimal and medial layers were carefully stripped and were dehydrated in cold acetone for 72 hours. The mucopolysaccharides were extracted by the method of Dyrbye and Kirk[10] with the following modifications: The original extract was precipitated directly with the acetate-alcohol precipitation mix. This obviated the necessity of heating the extract to reduce its volume. Also, unbound sulfate and phosphate were removed by dialysis against distilled water. The final precipitate was dissolved in water. Aliquots of this clear solution were then analysed. Total solids were determined gravimetrically, hexosamine by a modification of the method of Elson and Morgan,[13] hexuronic acid by the method of Dische.[9] Hydrolyzable sulfate was precipitated as the barium salt.

Hypercholesteremic serum was obtained from three rabbits on a diet of laboratory chow and egg yolk. Over a period of four months, a total of 21 different samples of sera were studied. Each sample was centrifuged at 78,000 g. for 30 minutes. An opalescent infranate was obtained with total cholesterol values between 500 and 1200 mg.%. In four experiments, this infranate was subfractionated by the method of Havel *et al.*[28] at densities of 1.019, 1.063, and 1.21. These infranates were dialysed against 0.9 per cent saline for 48 hours; the final volumes were adjusted to compensate for the previous dilution with the high density salt solutions. This procedure resulted in four serum subfractions from each original serum sample, with each successive fraction containing one less density subfraction: D. $>$ 1.006, D. $>$ 1.019, D. $>$ 1.063, and D. $>$ 1.21. The last sample, therefore, contained only lipid-protein complexes which did not rise at a density of 1.21.

For precipitation tests 0.1 ml. of serum was diluted to 1 ml. with an 0.2 per cent $CaCl_2$ solution. To this clear solution of diluted calcium-containing serum, 0.1 ml. of the mucopolysaccharide preparation was added. The pH of both the diluted serum and the polysaccharide solution was previously adjusted to 7.4 with dilute $Ca(OH)_2$. Precipitates developed immediately, and turbidity was measured with a Coleman Junior Spectrophotometer at a wave length of 680 mμ. These results were tabulated as follows:

optical density	
.01 - .04	±
.04 - .10	1+
.10 - .40	2+
.40 - 1.00	3+
1.00	4+

Lipid analyses were carried out by the methods routinely used in this laboratory.[20] Protein was estimated by the biuret technique, total nitrogen by the micro-Kjeldahl method.

The commercial preparation of chondroitin sulfate utilized was obtained as the potassium salt.* Part of this material was dialysed against 0.1 N acetic acid for three days, titrated to a pH of 7.4 with dilute $Ca(OH)_2$ and then redialysed against distilled water to remove the excess acetate salt. A final adjustment of pH was then made. A portion of the hyaluronic acid* was also dialysed and retitrated as the calcium salt. These solutions were then tested for precipitant activity by the usual test system.

Solutions of aortic mucopolysaccharide were incubated for 12 hours with bovine* and with bacterial** hyaluronidase. The precipitating activity of these solutions was then tested.

RESULTS

Two hundred fifty milligrams of polysaccharide-containing material was extracted from approximately 300 g. of aortic tissue, wet weight. Analysis of this material showed that hexuronic acid and hexosamine were present in equimolar ratios within experimental limits. Sulfate accounted for 11-12 per cent of the material. Analyses of two separate extracts are given (Table 1). Theoretical values for chondroitin sulfate are listed in column C. It is

TABLE 1. COMPARISON OF THE MATERIAL EXTRACTED FROM AORTIC TISSUE WITH CHONDROITIN SULFATE

	A *Extract I*	*B* *Extract II*	*C* *Chondroitin sulfate (calculated)*
Hexuronic acid	26%	25%	38%
Hexosamine	24%	22%	35%
Sulfate	11.8%	11%	19%
Protein	16%	12%	—
Ash	15%	11%	—

assumed that the hexuronic acid, hexosamine, and sulfate are present in equimolar proportions. On this assumption approximately 90 per cent of our preparation would be sulfated. Dyrbye and Kirk[11] report that approximately 80 per cent of their material was sulfated. Since no effort was made to remove nonpolysaccharide sulfate by differential precipitation in our procedure, this might account for the higher calculated values of sulfated mucopolysaccharide. The higher residual protein values of our material also reflect the milder conditions employed in this extraction. Since preservation as nearly as possible of the natural polymer was desired, and not chemical purity, these analytical compromises were considered justifiable.

When the extract was added to the test system, immediate and heavy precipitation was observed in all tubes containing serum of high lipid content. Tubes containing serum from rabbits on control low-fat, low-cholesterol diets remained clear. Dilutions of the extract gave detectable precipitates with serum of high lipid content down to a final mucopolysaccharide

* Nutritional Biochemical Corp., Cleveland, Ohio.
** Courtesy of Dr. Kenneth W. Thompson, Organon, Inc., Orange, New Jersey.

concentration of 2.0 μg. per ml., indicating the extremely active nature of this precipitating material (see Fig. 1).

Approximately 50 per cent of the total lipid was precipitated from six hyperlipemic rabbit sera from which the precipitate was analysed. Free and esterified cholesterol accounted for 30-45 per cent of the precipitate; triglyceride 20-26 per cent, and phospholipid 15-22 per cent. Small amounts of protein, 5-15 per cent, mucopolysaccharide, 2-3 per cent, and Ca 3-5 per cent, were also found (Table 2).

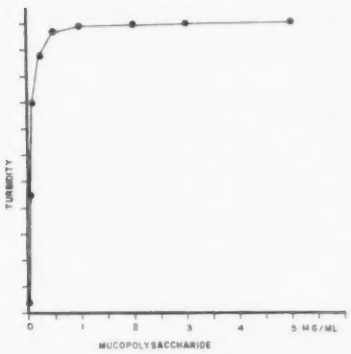

FIG. 1. Turbidity produced by addition of aortic mucopolysaccharides to dilute, hyperlipemic rabbit serum. Optical density x100 is plotted as turbidity on the vertical axis; final concentration of mucopolysaccharide, on the horizontal axis. Concentration of $CaCl_2$ remained constant at 0.2 gm.%; total lipid, in this sample: 1870 mg.%.

The precipitated complex was found to be extremely soluble in dilute solutions of NaCl. At a concentration of 5 mg. per ml. most of it was redissolved. To some extent, this could be reprecipitated by increasing the concentration of $CaCl_2$. However, $CaCl_2$ alone in excess of 10 mg. per ml. also redissolved the precipitate.

When the ultracentrifugally separated lipoprotein subfractions of the hypercholesterolemic sera were tested, only the infranates D. > 1.006 formed a heavy precipitate. Trace precipitation was noted in the D. > 1.019 infranates and there was no precipitation from the higher density infranates.

To determine whether this pattern of precipitation was a function of total lipoprotein concentration in the sample or of a specific lipoprotein floatation fraction, the density fractions were also separated by successive centrifugation. Only the 1.006-1.019 subfractions gave 4+ turbidity. The 1.019-1.063 subfractions gave ± to 1+ precipitation, in spite of the fact that this latter fraction contained more of the total lipid in two of the four samples which were subfractionated.

Defibrinated ACD human blood bank plasma also formed precipitates with the extracted polysaccharide. Total corrected cholesterol values of six samples ranged from 200 to 320 mg.%. In these precipitates, cholesterol and phospholipid were present in approximately equal amounts, comprising from 45 to 60 per cent of the precipitate. Triglyceride was usually present in small amounts; the Ca and mucopolysaccharide values corresponded to

those found with precipitates from rabbit sera. Protein made up 16 to 30 per cent of the precipitates. The total precipitable lipid was much lower, ranging from 5-15 per cent. Since lipoproteins in preserved plasma are liable to physical changes with aging, the above results were considered only as preliminary observations. This subject should be studied more fully with fresh human plasma.

At pH 7.4 commercial chondroitin sulfate mixed with hyperlipemic rabbit serum gave a precipitate of a lipoprotein-mucopolysaccharide complex only when the calcium salt of chondroitin sulfate was utilized. This material pre-

TABLE 2. ANALYSES OF MATERIAL PRECIPITATED FROM LIPID-RICH RABBIT-SERUM BY AORTIC MUCOPOLYSACCHARIDE

	Average of six samples	*Range of six samples*
Free cholesterol and cholesterol esters	41%	30-40%
Triglyceride	24%	20-26%
Phospholipid	17%	15-22%
Mucopolysaccharide	2.6%	2-3%
Calcium	3.9%	3-5%
Protein	14%	5-15%

1.0 ml. of aortic extract, containing 1.9 mg. of polysaccharide per ml. added to 1.0 ml. of serum diluted to 10.0 ml. with 0.2 per cent Ca Cl_2 solution.

cipitated serum lipoproteins down to a concentration of approximately 10 μg. per ml. The potassium salt of chondroitin sulfate exhibited no activity, even with added $CaCl_2$. Incubation of the mucopolysaccharide extracted from the aortic tissue with bovine hyaluronidase markedly diminished its precipitant activity. This effect was more pronounced with dilute solutions of the mucopolysaccharide. Bacterial hyaluronidase contrastingly had no effect.

DISCUSSION

The mucopolysaccharide extracted from aortic tissue by Dyrbye and Kirk[10, 11] was predominantly a form of chondroitin sulfate, with an additional smaller amount of nonsulfated mucopolysaccharide. Analyses of our material substantiates this finding. Since high temperatures and extreme pH values were intentionally avoided in our procedure, it would be expected that more firmly bound protein would remain with the mucopolysaccharide.[20] The mild conditions employed were designed to minimize depolymerization of the mucopolysaccharide.

In our test system the commercial mucopolysaccharide was active only if sulfate groups were present in the molecule. Hyaluronic acid showed no activity in concentrations up to 10 mg. per ml. In contrast lipoprotein precipitation was detectable at a chondroitin sulfate concentration of 10 μg. per ml. Experiments with other polymers corroborate this finding: sulfated dextran and sulfated amylopectin precipitate lipoproteins much more readily than do the nonsulfated parent compounds.[3, 23]

Dyrbye and Kirk[11] have demonstrated that sulfated mucopolysaccharide in the aorta is depolymerized by bovine hyaluronidase. Bacterial hyaluronidase was active only with the small amount of nonsulfated polysaccharide. Our results confirm this finding: after treatment with bovine hyaluronidase, the remaining polysaccharide fragments exhibited greatly diminished activity, whereas bacterial hyaluronidase had no effect upon the precipitant activity of the extracted mucopolysaccharides. This again suggests sulfated polysaccharide as the active material.

In morphological studies on human aortas, mucopolysaccharide accumulates in focal regions of elastic damage; lipid depostion occurs in these regions.[1, 13, 28, 29, 30] This polysaccharide is also depolymerized by bovine hyaluronidase and is unaffected by bacterial hyaluronidase.[28] From these studies, it has been suggested that this mucopolysaccharide is a form of chondroitin sulfate which collects in regions of intimal and medial damage and then in some way interacts with lipoproteins of the plasma to produce the focal lipid deposits.

In the present study the only lipoprotein floatation fraction that forms a heavy precipitate with the mucopolysaccharide is the D. 1006-1.019 fraction. It has been proposed that peptide and phospholipid groups form the hydrophilic portions of the beta lipoprotein molecule.[21] Although these hydrophilic groups form a relatively smaller percentage of the total molecule in the lower density fraction of the beta lipoproteins, the phospholipid decreases less than the peptide.[18] It would appear, then, that the 1.006-1.019 fraction would be more dependent on its phospholipid for solubility than would the higher density lipoglobulins. Ca ions added to a solution of lecithin will immediately precipitate a Ca-lecithin complex,[5] suggesting that the phospholipid in the lipoprotein molecule also may be binding Ca ions. Some of the chondroitin sulfates in tissue exist as the Ca salt. Work by Boyd and Neuman[4] suggests that Ca ions are strongly bound by the sulfate groups in chondroitin sulfate; phosphate can then be bound to this Ca-chondroitin sulfate complex. Thus, one possible mechanism binding mucopolysaccharide to lipoprotein in an insoluble complex would be through the mutual binding of the Ca ions by the macromolecules. This mechanism would explain the specificity of the

precipitation, including the necessity for sulfate groups on the polysaccharide and for phospholipid in lipoproteins with low peptide content. Extraction of lipoproteins from aortic tissue has shown the bulk of the lipid to be associated with this low density beta lipoglobulin fraction.[15]

The redissolving of the mucopolysaccharide-Ca-lipoprotein precipitate by relatively high concentrations of $Ca(Cl)_2$ and lower concentrations of NaCl is probably due to a dissociation of the complex.[21] A shielding of ionic sites in polyions by oppositely charged ions has been demonstrated by Fuoss;[14] this effect becomes more intense at higher ionic concentrations. This suggests that the electrostatic forces of attraction between the oppositely charged macromolecules would be decreased; further, with added Na ions competing with the Ca ions for the available sites on the macromolecules, the net binding effect of the bivalent Ca ions would be greatly diminished. Such a competitive mass action effect can be demonstrated experimentally at low concentration of $Ca(Cl)_2$: addition of a small amount of NaCl to the test system will markedly decrease the precipitate. Further addition of $CaCl_2$ will reform a heavy precipitate. Eventually an upper limit is reached, at which further addition of $CaCl_2$ will no longer reprecipitate the complex. It may be that in this range the shielding effect of the high Cl ion concentration becomes dominant.[21]

This inhibitory effect of NaCl in physiological concentrations indicates that if lipoproteins are bound by a Ca salt of chondroitin sulfate in tissue, other factors of equal importance must be considered. It must be remembered that the intima is a complex structure of collagen and elastic fibers, a ground substance of polysaccharide material, and an endothelial covering, as well as substances possibly filtering from the plasma. A discussion of concentration in such a multi-phase system is almost meaningless. For example, in focal regions of injury, degradation products produce a gel-like mass of metachromatic material. In such a region, a lipid-rich precipitate from plasma would be favored by the high concentration of macromolecular substances[21, 23] by means of a "dehydration" mechanism; any accumulation of sulfated polysaccharides would also favor lipoprotein precipitation. Thus, the interplay of different factors in any small volume of intima would determine the solubility of the lipoprotein in this region.

By adding heparin to a solution of serum dialysed to a low ionic strength, Burstein[7] has precipitated virtually all of the beta-lipoprotein fraction. No precipitation occurs unless Ca ions are added to this system. Although heparin is a relatively small polysaccharide, it has a relatively high sulfate content.

$CaCl_2$ in low concentrations also increases the precipitation of serum lipoproteins by solutions of polyethylene glycol;[3] higher concentrations of the Ca ion will redissolve the precipitate. Polyethylene glycols are known chelating agents, binding Ca ions strongly; this again suggests that the Ca ion is the bivalent intermediate between these two macromolecules. Since high concentrations of these polymers will precipitate serum proteins without additional Ca ions,[29] nonionic forces may also be involved.

Walton[27] has shown a similar binding of fibrinogen with dextran sulfate at physiologic pH; the importance of nonionic forces in these reactions was stressed, in contrast to the ionic binding which occured at pH levels below the isoelectric point of fibrinogen. He also noted the extreme solubility of these precipitates in dilute NaCl solutions at physiologic pH. Studies by Smith and Van Korff[25] have demonstrated a slow precipitation of fibrinogen from heparinized plasma. In one experiment in the present study, utilizing a solution of fibrinogen in place of the serum lipoprotein in the test system, a heavy precipitate formed with the addition of the aortic mucopolysaccharide. This precipitate also redissolved with excess $CaCl_2$ or NaCl. The redissolved precipitate clotted when thrombin was added.

Size of the reacting molecules appears to be of great importance in the type of reaction under discussion. The larger polymers of polyethylene glycol are much more active, weight for weight, than the shorter polymers in the precipitation of lipoproteins.[3] Walton[27] also noted that only the larger sulfated polysaccharides would precipitate fibrinogen at physiologic pH. Bernfeld[8] also observed this phenomenon with sulfated starches. Cohen[5] found that only the larger polysaccharides were effective in the precipitation of virus particles. In our experiments the depolymerized material, after treatment with bovine hyaluronidase, showed greatly diminished activity. Large size may also be a factor in the specificity of the lipoprotein precipitation, for the 1.006-1.019 density fraction approaches the chylomicron of the plasma in size. In the tissue the problem is further complicated by a complex structuring in which molecules are rigidly positioned. Such structural rigidity may produce a simple filtering and dehydration of the macromolecules, without even the necessity of macromolecular binding.[21, 33, 34]

SUMMARY

Sulfated mucopolysaccharides extracted from the intima and media of the human aorta will precipitate lipoproteins of diluted human serum at a pH 7.4.

The most precipitible lipoprotein is the D. 1.006-1.019 fraction. The D. 1.019-1.063 fraction shows some precipitation. Ca ions in low concentrations are necessary for precipitation.

Ca ions, mucopolysaccharide, and lipids and protein are found in the precipitated complex.

The Ca salt of a commercial chondroitin sulfate has similar precipitant activity, while hyaluronic acid does not. Bovine hyaluronidase diminishes this activity, while bacterial hyaluronidase has no effect.

An electrostatic binding mechanism is suggested, involving Ca ions, the sulfate groups on a form of chondroitin sulfate, and the phospholipid of the lipoproteins. Other forces, related in part to molecular size, must also be involved.

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The Board of Editors

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THE PRECIPITATION OF SERUM LIPOPROTEINS BY GELATIN†

A number of studies have recently demonstrated a selective precipitation of the lipoglobulins of human serum by substances of high molecular weight; these include sulfated dextran,[12] heparin with phenol,[10] sulfated amylopectin,[8] agar agar,[8] polymers of ethylene glycol,[14] and polyvinyl alcohol.[1] Except for the study of Bernfeld *et al.*[2] relatively little investigation of the precise mechanism of these reactions has been made.

The demonstration of macromolecules in the connective tissue of the walls of arteries in chronic vascular disease, the possibility that lipoproteins of serum may be present in such sites,[9, 14, 15] and the presence of gel-like masses of metachromatic material,[5] suggested to us that precipitation reactions between serum lipoproteins and macromolecules might also take place intramurally in arteries and account for deposition of lipids there. Particularly in regions of injury, metachromatic materials and degraded products of collagen might be expected to be present. In view of these considerations, the interactions of various macromolecules with human plasma proteins are being investigated *in vitro*. As preliminary trials of mixtures of normal human plasma in gelatin solutions led consistently to lipid-rich protein precipitates, these reactions were selected for initial study.

MATERIALS AND METHODS

Serum of high lipid content was obtained from rabbits on a diet of laboratory chow supplemented by egg yolk. Total cholesterol values of these sera ranged from 300 mg. to 1000 mg. per cent. High-lipid plasmas were selected from recently outdated ACD whole bank blood. Since fibrinogen precipitated and on occasion formed fibrin clots in the test system, these plasmas were defibrinated with thrombin prior to use. Corrected cholesterol values for the plasmas ranged from 200 mg. to 350 mg. per cent.

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Eastman Purified Calfskin Gelatin with an isoelectric point of 4.8 was used. In some experiments requiring large quantities of gelatin, a less pure commercial preparation was utilized with no significant differences in results. The gelatin was dissolved in water by heating to 95° C., and the solution was then filtered clear under suction. Solutions up to 20 per cent were found to be workable. At high concentrations, the viscosity at 37° C. made manipulation difficult.

Optical density measurements were made on a Coleman Junior Spectrophotometer at a wave length of 680mμ. Lipoprotein fractions were prepared in the Spinco Model L preparative ultracentrifuge, using the method of Havel *et al.*[10] The lipid analyses

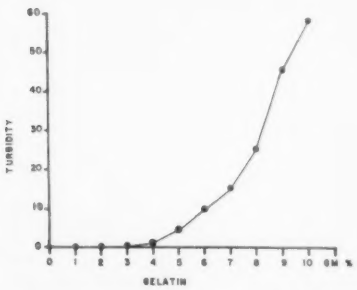

FIG. 1. The precipitation of plasma lipoglobulins by addition of gelatin. Two ml. of plasma added to 2 ml. of the gelatin solutions. The final concentration of gelatin is given on the horizontal axis; the resultant optical density (x100) plotted as turbidity on the vertical axis. Total plasma lipid of this sample: 714 mg.%.

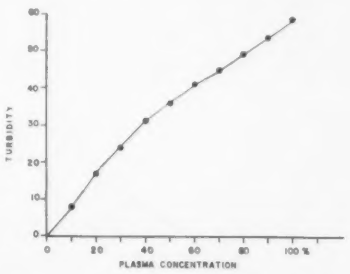

FIG. 2. The precipitation of lipoglobulins of diluted plasma by gelatin. Two ml. of a 20 per cent gelatin solution added to 2 ml. of diluted plasma. The proportion of plasma in the 0.9 per cent saline-plasma solution is given on the horizontal axis; the resultant turbidity on the vertical axis. Total plasma lipid of this sample: 714 mg.%.

were performed with the methods routinely in use in this laboratory.[16] In addition, the lipids in precipitates were determined by the method of Bragdon.[4] Determinations of protein were by the usual micro-Kjeldahl technique and in some instances by the biuret method.

All mixing of reagents was performed at approximately 37° C.; readings were made within ten minutes. Prior to mixing, the pH of each solution was adjusted to 7.4 with dilute HCl and NaOH, unless otherwise indicated.

EXPERIMENTAL RESULTS

Equal volumes of gelatin solutions and serum or plasma were mixed at 37° C. A lipid-rich precipitate formed with a final gelatin concentration of approximately four per cent; this became progressively greater with increasing gelatin concentrations (Fig. 1). In testing 12 samples with total lipids ranging from 500 mg. per cent to 1500 mg. per cent, it was found that the point of first precipitation and final amount of precipitate varied roughly with the total lipid in the sample. No precipitate formed with control rabbit

serum at pH 7.4. With varying plasma or serum concentration, however, the slope remained nearly constant; the gelatin concentration was kept constant at 10 per cent (Fig. 2).

Four samples of serum and plasma were fractionated by centrifugation at 75,000 g. for six hours at densities 1.006, 1.019, 1.063, and 1.21. All infranates were dialysed against 0.9 per cent NaCl solution, a step that was found necessary because high concentrations of NaCl and KBr sharply

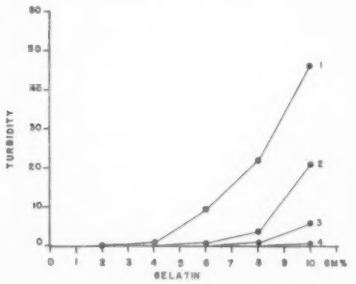

FIG. 3. The precipitation of lipoglobulins from plasma infranates after centrifugation at 78,500 g. for six hours. 1. Infranate at d. 1.006 (82 per cent); 2. infranate at d. 1.019 (69 per cent); 3. infranate at d. 1.063 (29 per cent); 4. infranate at d. 1.21 (8 per cent). Two ml. of plasma infranate mixed with 2 ml. of gelatin solutions. Final gelatin concentration is given on the horizontal axis; resultant turbidity, on vertical axis. Total plasma lipid of this sample: 890 mg.%. Lipid in each infranatant fraction, stated as percentage of original lipid concentration, is given in parenthesis.

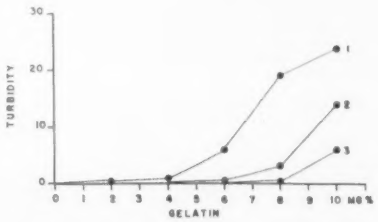

FIG. 4. The precipitation of lipoglobulins from solutions of ultracentrifugally separated lipo-proteins. Density fractions are: 1. 1.006-1.019, 2. 1.019-1.063, 3. d. 1.063-1.21. Two ml. of lipoprotein solution mixed with 2 ml. of gelatin solutions. Final gelatin concentration is given on horizontal axis; resultant turbidity on the vertical axis. Total plasma lipid of this sample: 890 mg.%.

decreased the amount of precipitate in the gelatin-plasma or gelatin-serum solution. Volumes were then adjusted to contain equal amounts of the original material and mixed with a similar volume of a 20 per cent gelatin solution. The d. 1.006 infranate yielded the heaviest precipitate at all concentrations of gelatin studied (Fig. 3). There was a progressive decrease in turbidity with the removal of the higher density lipoprotein fractions; no significant precipitation was found in the virtually lipid-free d. 1.21 infranate. The lipoglobulins were also isolated into three density fractions, d. 1.006-1.019, d. 1.019-1.063, and d. 1.063-1.21, dialysed against 0.9 per cent NaCl, and mixed with gelatin solutions. Experimental curves (Fig. 4) were obtained which were similar to the plots of the differences between the curves

shown on Figure 3; in general, the d. 1.006-1.019 fraction began to precipitate at lower gelatin concentrations and levelled off at higher gelatin concentrations.

Precipitate formed within a wide range of pH values, from 9 down to extremely acid values. As pH decreased below 7.4, precipitation increased in the higher density infranates. Below pH 5.5 in all fractions, including the d. 1.21 infranates and control rabbit sera, there was heavy precipitation. On analysis this material proved to be protein.

The precipitate formed at pH 7.4 with plasma or serum in 10 per cent gelatin was extremely soluble in water and saline solutions; it was, therefore, washed free of nonprecipitated lipid with a concentrated gelatin solu-

TABLE 1. VALUES OF LIPIDS IN A SAMPLE PLASMA AND OF LIPIDS PRECIPITATED IN A 10 PER CENT GELATIN

Lipid fraction	*Mg. Lipid/10 ml. plasma*	*Mg. Lipid precipitated from 10 ml. plasma*
Fatty acids	55.7	12.3
Total cholesterol	22.2	6.5
Phospholipid	17.3	4.8

tion. The total lipid precipitated varied to some extent with the lipid concentration of the original solution; the heaviest precipitates were obtained from rabbit sera of high-lipid content. In eight analyses, the composition of the precipitate varied according to the original composition of the plasma or serum; cholesterol, phospholipid, and fatty acids were found in all precipitates. A typical analysis is given in Table 1. Since the precipitates were insoluble only in the presence of high concentrations of gelatin at pH 7.4, no protein analyses were performed.

DISCUSSION

The precipitation of serum proteins by gelatin solutions appears to be selective for lipid-rich globulins at pH 7.4; with decreasing pH other serum proteins precipitate. The data suggest that at pH 7.4 the large, low density lipoprotein fractions are most susceptible to precipitation, especially at the lower concentrations of gelatin. The mechanism of precipitation does not appear to be a simple binding of oppositely charged poly-ions. If this were the case, precipitation would be expected only in the narrow range between the isoelectric points of the proteins involved. Bernfeld[3] has noted, however, that lipoprotein binding occurs outside this inter-isoelectric range with a

number of sulfated polysaccharides. Solubility of the resulting complex was a function of the size and structure of the poly-ion, and of the particular type of functional ionic groups present.

The shape of the curve in Figure 1 resembles the curves obtained when plasma proteins are precipitated with salts[5] or cold alcohol;[6] in such instances there is no precipitation until a critical concentration of the added precipitant is reached. This process involves the rearrangement of water molecules away from the protein.[7] The extremely high viscosities of the concentrated gelatin solutions suggests that the bulk of the water may be hydrating the gelatin molecules.[11] Also, in the cold alcohol methods, the lipoglobulins are among the first fractions to precipitate.[6] This would suggest that the high concentration of gelatin serves to dehydrate the plasma proteins to the point where solubility of the larger, low density lipoglobulins cannot be maintained. In a floatational analysis of the lipoproteins of the aorta, Hanig found most of the lipoprotein present to be of this low density fraction.[9]

SUMMARY

Lipoproteins are selectively precipitated from serum and from defibrinated plasma by concentrated gelatin solutions at pH 7.4. The larger, low density lipoproteins are precipitated at low gelatin concentrations. Higher gelatin concentrations are necessary for precipitation of higher density lipoproteins.

Precipitation occurs at a wide range of pH values; nonlipid plasma proteins begin to precipitate at pH values below 7.4. This precipitation range argues against a simple electrostatic attraction between two oppositely charged poly-ions.

The precipitation curves with gelatin resemble the precipitation curves for the "salting out" of plasma proteins.

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AN EFFECT OF AGGREGATION ON THE BEHAVIOR OF A BEETLE (*CONOPHTHORUS CONIPERDA* Sz) IN A TEMPERATURE GRADIENT

The white pine cone beetle (*Conophthorus coniperda* Sz., Scolytidae) infests the twigs and cones of white pine throughout its range in eastern North America. The insects pass the winter in the adult stage within abortive cones on the forest floor. In spring, the adults emerge and move to the tops of the trees where they infest the current cone crop. Eggs are deposited in the growing cones and the life history is completed within the brood cones during the period from early spring to midsummer.[3]

The only period in its life history when the insect is free-living and active is during the move from the abortive brood cones on the forest floor to the cones of the subsequent crop. This situation is particularly interesting in that the entire dispersive and distributional pattern of the insect must be established for each generation within a very short period. This report is concerned with a behavioral mechanism which is probably of considerable importance in determining the distribution of the insect.

MATERIALS AND METHODS

Initial experiments were done in which groups of insects were placed on fine nylon net above an array of salt solutions providing a gradient of humidity. These were supplemented by the exposure of groups of insects to a rising draft of air at graded velocity which gave a gradient in evaporation rate. Adults of *Conophthorus coniperda* on emergence from moist cones within which there was access to free water are rather indifferent to gradients of both humidity and evaporation rate. This finding permits one to expose insects to gradient temperatures with some confidence that the resulting movements of the insect are in fact modified by temperature and not by the obvious correlevant gradients of evaporation rate and humidity.

During preliminary experiments, the standard technique was to place a group of insects in a temperature gradient apparatus.[6] A heavy copper plate was heated at one end and cooled at the other by streams of water under thermostatic control. Surface temperatures to which the insects were exposed were monitored with thermocouples built into wells let in the plate. The machine was designed to offer a range of temperatures from 10° C. to 40° C. Insects were held in the gradient for an hour at which time the temperature of the location adopted by each insect was noted. A con-

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siderable between-group variation in the temperature chosen was found though the within-group variation was low. This was surprising since the groups were taken from a pooled stock with elaborate precautions to avoid bias. Some variation in the size of the groups used had been permitted during preliminary experiments. It was noticed that the larger groups tended to display a higher temperature choice. A series of experiments was designed to investigate this observation.

RESULTS

The behavior of a series of groups ranging in size from 10 to 100 insects was determined in the standard manner. The results are presented in Figure 1. It will be seen that the lower limit of temperature was independent of

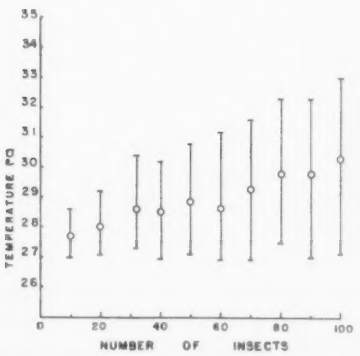

FIG. 1. Means and ranges of temperatures adopted by groups of *Conophthorus coniperda* Sz.

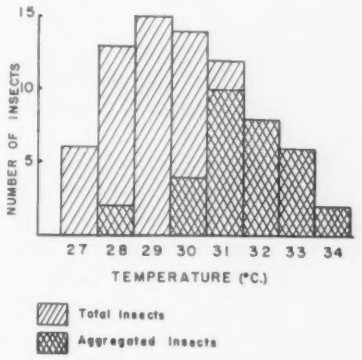

FIG. 2. Temperatures adopted by aggregated and unaggregated *Conophthorus coniperda* Sz.

density but that the mean and upper limit of temperature adopted increased with density.

During the course of these experiments, a further peculiarity of the behavior was noticed. Particularly in the upper part of the gradient and particularly at high densities, a great many of the insects were aggregated. The form of the aggregation was a very tight clumping of from 2 to 20 individuals. The insects within these clumps appeared to move very little if at all. Sex determinations by dissection revealed no sexual bias in the makeup of a number of aggregations.

In Figure 2, the over-all distribution of individuals affected by gradient temperature after one hour of exposure is shown with the position of aggregations indicated. It is apparent that the aggregated individuals occupy a temperature range which is distinct from that of the unaggregated insects.

The mean temperature chosen by the aggregated insects is 31.5° C. The mean temperature chosen by unaggregated insects is 28.6° C. All the insects in the upper part of the gradient were aggregated.

Since the aggregated insects apparently behave in a manner rather different from the nonaggregated, a series of tests was run with single insects in the gradient. Each insect was exposed for an hour and its final position recorded. The mean temperature adopted by these single insects was 28.5° C. which is the same mean temperature found in the smallest groups in the tests illustrated in Figure 1.

There is no reason to suppose that the insects tested singly had any inherent difference from those tested in groups. It seems most likely that the different behavior of the latter results from their opportunity to form aggregations which, once formed, are immobile.

When the positions of single insects in the gradient were recorded at five-minute intervals for two hours, the unexpected pattern shown in Figure 3 was obtained. Apparently the insects, on introduction to the gradient, range widely. At first they tend to adopt a high temperature, but as time passes the temperature adopted and the range over which the insects move decrease. Thus after 20 minutes in the gradient, the mean temperature chosen was 32° C. while after an hour, the mean temperature was 28.5° C. Interestingly enough, the distribution after 20 minutes is identical to the distribution of aggregated insects shown in Figure 2.

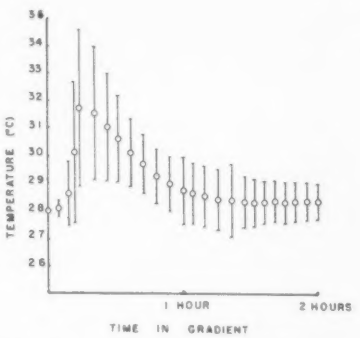

FIG. 3. Means and standard deviations of temperatures adopted after periods up to two hours in the gradient apparatus.

Direct observation suggests that when two insects meet, or when a single insect collides with a group, a new group is formed. These clumps do not move and very seldom separate. Groups of insects were held in standard containers at controlled temperatures. If density were constant, the number of insects in aggregations increased with time but was independent of temperature when the time allowed was over an hour.

For any given time and any level of temperature the number aggregating was a direct function of density. For times up to 30 minutes, at any given density level, the numbers of insects in aggregations was a direct function of temperature. Walking speed is a direct function of temperature when the

insects are tested during their orientation to a point source of light. It was not possible to demonstrate that the track of a light oriented insect was influenced by the close presence of tethered insects. However, a tethered insect directly in the path of an orienting insect formed the basis of a stable aggregation following collision.

These results suggest that the probability of an insect entering an aggregation is simply the probability of a collision with another insect or with an aggregation. The reason these aggregations are most frequent at higher temperatures at any given time lies in the greater walking speed and higher probability of collisions at high temperatures. As the probability of collision is affected by density as well as by walking speed, collisions become less frequent after insects have been in the gradient for some time. This results from the fixing of a substantial portion of a group of insects within aggregations that are formed in high temperature zones soon after the insects are introduced into the gradient. As time passes, the unaggregated insects move down the gradient and away from the fixed portion of the group. This, in effect, lowers the density.

When insects are first introduced into the gradient, they move to rather high temperatures. As time passes, the temperatures chosen are lower. Other experiments suggest that desiccation of some insects before testing results in lower initial preferenda.[4] From this, I would suspect that the *Conophthorus* in the present study move down the gradient as they lose water. Aggregated insects continue to occupy positions in the gradient which would be quite untenable if they were not fixed by their aggregation.

The aggregation of *Conophthorus* following chance encounter is not at all well understood. It seems to be mostly a thigmotropism since individual insects may be fixed by grains of rice, wood chips, or a variety of other objects, some quite unlike beetles in shape and color. The insects may also be fixed under filter paper shelters and in contact with a large vertical obstruction such as the wall of the chamber. In general, the insects seem to move in a way which brings them into maximum physical contact with their surroundings.[1] They seldom move in a manner which reduces the amount of such contact.

This mechanism suggests a manner in which aggregations of insects which lack integrating mechanisms of a social sort might be maintained. A case in point would be the very stable aggregations of *Coccinellidae* which are seen in unusual situations at times.[2] Perhaps such aggregations could be investigated by techniques similar to those used in this study.

In the case in hand, the reactions involved probably have a most important part to play in the ecology of the insect. While the insects are free moving

and before they enter cones of the current crop, they would move away from the exposed parts of the crowns during times of full insolation when the temperatures of the tree surfaces would certainly rise above their preferendum. However, after the insects have entered the cones, they would tend to be fixed by their proximity to other animals and by the high degree of physical contact with the cones in which they were feeding. Under such circumstances, temperatures considerably higher than the preferendum would be endured without beetles leaving their cones in search of more favorable temperature conditions. In other words, the experiments reported here demonstrate a mechanism through which a reaction is suspended but not lost. The masking effect of the thigmotropism can apparently prevent a strongly thermotaxic animal from being dislodged from its habitat by unfavorable temperature experience.

SUMMARY

1. *Conophthorus coniperda* Sz. in an open arena forms thigmotropic aggregations which are stable and immobile.
2. These aggregations are formed as a sequel to accidental collision and thus are more frequent at high density and when speed of movement is high at high temperatures.
3. In a temperature gradient, aggregation is most frequent at high temperatures. The aggregations are formed while the insects are in hot portions of the gradient early in their exposure. The aggregated insects are immobilized in a part of the gradient hotter than that finally adopted by unaggregated insects.
4. The thigmotropic response has the effect of holding strongly thermotaxic insects immobile during exposure to periods of field temperature above the preferendum.

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OBSERVATIONS ON THE EFFECT OF EXERCISE ON BLOOD AMMONIA CONCENTRATION IN MAN†

In 1925 Luck, Thacker and Marrack reported elevated blood ammonia levels in epileptic patients after grand mal seizures.[19] Although they suspected that muscular exercise might be responsible for this rise, they apparently excluded this possibility by their inability to produce increased venous ammonia levels by vigorous muscular exercise. Several years later Parnas and his co-workers noted that the blood ammonia concentration was elevated in the veins which drained the exercising forearms of normal subjects.[23] These investigators demonstrated that ammonia liberated by the muscle during fatiguing exercise was responsible for this rise. These observations were confirmed by Kalk and Bonis.[17] Recently, Schwartz, Lawrence, and Roberts re-investigated some aspects of this problem.[24] They induced convulsions in dogs and observed an increase in the arterial ammonia concentration which could be prevented by suppressing the motor aspects of the convulsions with *Anectine*℞. In addition, they were able to produce increased systemic blood ammonia levels in normal men by vigorous generalized exercise.

The recognition by Bessman and his co-workers of the importance of the peripheral tissues in ammonia metabolism has given fresh significance to these earlier observations. These investigators showed that as the arterial ammonia concentration rises in patients with hepatic coma, the peripheral tissues extract correspondingly large amounts of ammonia.[3, 4] These findings which were based on simultaneous arterial and venous ammonia determinations have been confirmed[20, 30, 34] and extended to show that in the terminal phases the peripheral tissues may sometimes liberate ammonia into the systemic circulation.[20, 34] Similar observations have been made following the administration of ammonium salts to normal subjects and animals.[1, 13, 31] The significance of the uptake of ammonia by the peripheral tissues, presumably muscle, is not clear. It has been suggested that this extraction of am-

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† The material for this paper is taken from a thesis submitted in 1959 by Dr. Allen to the Faculty of the Yale University School of Medicine in partial fulfillment of the requirements for the degree of Doctor of Medicine.

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monia protects more vulnerable sites from high ammonia concentrations. Furthermore, acetazoleamide (Diamox ℞), which may induce impending hepatic coma in cirrhotic patients, has been shown to decrease the arterial-venous ammonia difference and may thus interfere with such a protective function.[11]

This investigation was undertaken to evaluate the effects of muscular exercise on the release of ammonia from peripheral tissues, to compare these phenomena in normal subjects and in cirrhotic patients, and to further our understanding of these phenomena.

MATERIALS AND METHODS

The subjects of these investigations comprised three groups: 10 normal male volunteers, 31 male ambulatory patients with a variety of benign, nonhepatic disorders, and 17 male ambulatory patients with histologically proven cirrhosis.

The blood ammonia* values were determined in triplicate according to the method of Seligson and Hirahara[25] within ten minutes of collecting the blood. Blood was drawn without stasis into 20 ml. syringes which had been coated with heparin. Venous blood was withdrawn from an antecubital vein through an indwelling needle after a small specimen of blood had been discarded. Arterial blood was obtained from either the brachial or femoral artery.

The normal venous blood ammonia concentration, which was determined on ten normal subjects, was 161 ± 21 μg. NH_3 N per 100 ml. This normal range, which was obtained with Upjohn Co. heparin (1000 units per ml.), is much higher than our previous normal range (102± 23)[9] which was established with Organon, Inc. heparin as anticoagulant. It was demonstrated, in retrospect, that these higher normal values were caused by the greater ammonia content of the Upjohn heparin solution. These observations were the subject of a separate communication on the effect of different heparin solutions on the blood ammonia determination.[9] A single lot of Upjohn heparin (1000 units per ml.) was used throughout this study.†

Blood oxygen saturation was determined by the method of Van Slyke and Neill.[28] The hydrogen ion concentration of blood was measured with a Cambridge Model R pH meter. Serum carbon dioxide, sodium, chloride, potassium, magnesium, and glutamic oxalacetic transaminase (SGOT) were determined according to standard methods previously employed in this laboratory.[7, 8]

All subjects were studied in the sitting or supine position after resting for 15 minutes. None had had any vigorous muscular exertion prior to examination and none was allowed to squeeze or clench his fist prior to or during the blood collection. Mild exercise consisted of clenching the fist vigorously one hundred times at a rate of one per second. A spring-type hand grip exerciser ("Power Gripper") was em-

*Although it is recognized that ammonia exists almost entirely as ammonium ion at the pH of blood, the term blood ammonia is used throughout this paper to represent the total ammonia-ammonium content of blood.

† There were small variations in the amount of heparin required to wet the barrels of individual syringes (0.14 to 0.18 ml.). Such variations could cause small fluctuations in blood ammonia levels. This study, however, is concerned primarily with relative, rather than absolute, blood ammonia values, and the random distribution of this artifact among the experimental determinations permits valid statistical analysis.

FIG. 1. "Power Gripper" used to exercise the hand and forearm muscles.

ployed to exercise the subjects more strenuously (Fig. 1). Fifty "power grips" at the rate of one per second were considered moderate exercise. One hundred such "power grips" (defined as severe exercise) were sufficient to fatigue the forearm muscles of all normal subjects.

Acetazoleamide (250 mg.) was given orally 13 hours and 1 hour prior to studying the effect of muscular exercise on the blood ammonia concentration in a group of cirrhotic patients in whom baseline exercise studies had previously been made.

RESULTS

Mild exercise. In 10 control patients and 7 cirrhotic patients venous ammonia samples were drawn from the exercising arm before and immediately after 100 fist clenches. The results are shown in Table 1 and Figure 2.

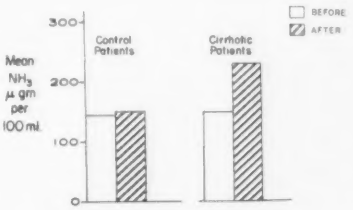

FIG. 2. A comparison of the effect of mild exercise (100 fist clenches) on the mean venous ammonia levels in the exercising arm in cirrhotic and non-cirrhotic patients.

Five cirrhotic patients showed elevations in excess of 40 μg. per 100 ml. and 2 showed no appreciable change. Only 1 patient in the control group showed a large rise after exercise and 1 showed a moderate fall. The difference between the two groups is highly significant ($p < 0.01$). Although the resting blood ammonia level was higher in the cirrhotic patients, there was no correlation between the initial blood ammonia concentration and the absolute or percentage rise observed.

Moderate exercise. In 6 control patients and 7 cirrhotic patients venous ammonia concentrations were determined before and immediately after 50 "power grips." The results are shown in Table 1 and Figure 3.

There was a mean rise of 5 μg. per 100 ml. in the control group and of 80 μg. per 100 ml. in the cirrhotic group. Although 2 of the control patients showed increases of 35 and 36 μg. per 100 ml., respectively, all 7 cirrhotics exhibited increments greater than 45 μg. per 100 ml. The difference between the two groups is highly significant ($p < 0.001$).

Severe exercise. The effect of 100 "power grips" was compared in 10 normal subjects, 10 control patients, and 10 cirrhotic patients.

The mean venous blood ammonia values before exercise were not significantly different in the three groups. All 30 subjects showed large increments in the blood ammonia concentration after exercise. The difference in

response among the three groups is not statistically significant (Table 1, Figure 4).

In addition to these measurements blood ammonia determinations were made 15 minutes after the termination of exercise in some of the subjects in each group. The mean venous ammonia level was still significantly elevated 15 minutes after exercise although several patients in each of the three categories had returned to the resting level (Table 2).

Simultaneous venous blood specimens were also obtained from the resting arm before, immediately after, and 15 minutes after exercise in some of the

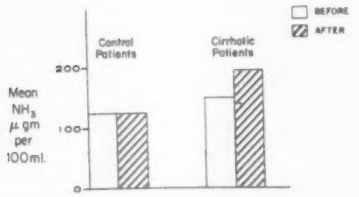

FIG. 3. A comparison of the effect of moderate exercise (50 "Power Grips") on the mean venous ammonia levels in the exercising arm in cirrhotic and non-cirrhotic patients.

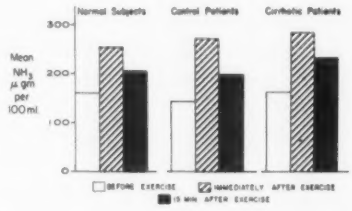

FIG. 4. A comparison of the effect of fatiguing exercise (100 "Power Grips") on the venous ammonia concentration in normal subjects, cirrhotic patients and non-cirrhotic patients.

subjects in each group. These ammonia levels did not change significantly from the resting levels (Table 2).

In 3 control patients and 2 cirrhotic patients both arterial and venous ammonia concentrations were measured before and after 100 "power grips." In each of the 5 patients large increments in the venous ammonia levels were observed after exercise with a mean increase of 121 μg. per 100 ml. However, the mean increment in the arterial ammonia concentration was only 11 μg. per 100 ml. This rise was not statistically significant. In each instance the venous ammonia value was much higher than the arterial concentration. This negative arteriovenous difference, which ranged from -66 to -154 μg. per 100 ml., was not related to either the pre-exercise arterial or venous ammonia concentrations.

Nine cirrhotic patients in whom the effect of severe muscular exercise on blood ammonia had been studied were re-studied within one week to evaluate the effect of acetazoleamide on this phenomenon. Conditions were otherwise unchanged. There was no significant difference in response after acetazoleamide from the results obtained previously. Each patient, both

before and after this drug, showed a sharp rise in venous ammonia concentration. It is interesting to note the similarity of response of the individual patients on the two occasions (Table 3).

TABLE 1. VENOUS BLOOD AMMONIA BEFORE AND AFTER EXERCISE

	Mean and standard deviation Venous NH_3 N in μg. per 100 ml.		
Mild Exercise	*Resting*	*After exercise*	*Difference*
Control patients (10)	127 ± 21	127 ± 24	0 ± 20
Cirrhotic patients (7)	151 ± 26	195 ± 47	43 ± 22
Moderate exercise			
Control patients (6)	146 ± 6	151 ± 30	5 ± 24
Cirrhotic patients (7)	148 ± 15	229 ± 21	80 ± 35
Severe exercise			
Normal subjects (10)	161 ± 12	254 ± 64	94 ± 69
Control patients (10)	144 ± 51	272 ± 82	128 ± 87
Cirrhotic patients (10)	163 ± 44	286 ± 54	122 ± 49

TABLE 2. VENOUS BLOOD AMMONIA BEFORE, IMMEDIATELY AFTER AND 15 MINUTES AFTER SEVERE EXERCISE

	Mean and standard deviation Venous NH_3 N in μg. per 100 ml.		
Exercising arm	*Resting*	*Immediately after exercise*	*15 Minutes after exercise*
Normal subjects (8)	157 ± 13	259 ± 70	202 ± 65
Control patients (8)	143 ± 24	230 ± 86	200 ± 51
Cirrhotic patients (6)	190 ± 58	300 ± 58	236 ± 70
Resting arm			
Normal subjects (7)	164 ± 29	167 ± 28	156 ± 26
Control subjects (5)	163 ± 15	159 ± 20	158 ± 18
Cirrhotic subjects (5)	170 ± 53	179 ± 45	175 ± 32

In a group of 5 control patients and 4 cirrhotic patients venous blood samples were obtained for blood pH, oxygen saturation, serum carbon dioxide content, sodium, chloride, potassium, and magnesium before and immediately after 100 "power grips." These determinations were performed in order to evaluate the relationship of changes in hydrogen ion concentra-

tion, oxygen saturation, carbon dioxide content, and serum electrolytes to the increase in blood ammonia concentration (Table 4).

All 9 patients exhibited a fall in venous blood pH following exercise. The mean pH fell from 7.40 to 7.28. This change, which is highly significant ($p < 0.001$), was greater in the cirrhotic than in the control patients.

TABLE 3. EFFECT OF ACETAZOLEAMIDE ON THE VENOUS AMMONIA CONCENTRATION AFTER 100 "POWER GRIPS" IN CIRRHOTIC PATIENTS

Cirrhotic patients	*Resting*	*After exercise*	*Difference*
	Before Diamox		
W.C.	135	258	123
C.M.	279	330	54
S.S.	197	275	78
L.S.	116	232	116
J.S.	139	207	68
D.S.	104	305	201
J.W.	130	310	180
J.K.	167	313	146
W.P.	143	291	148
Mean ± S.D.	156 ± 55	280 ± 42	124 ± 40
	After Diamox		
W.C.	145	261	116
C.M.	256	285	29
S.S.	215	225	10
L.S.	151	370	219
J.S.	161	235	74
D.S.	108	269	161
J.W.	147	314	167
J.K.	211	324	113
W.P.	138	281	143
Mean ± S.D.	170 ± 48	285 ± 59	115 ± 65

All of the patients showed a rise in venous carbon dioxide content after exercise. The mean serum CO_2 rose from 23.0 to 25.1 mEq. per L. This change is highly significant ($p < 0.001$).

The degree of oxygen saturation decreased in 6 of 8 subjects, including the 5 control patients. In one cirrhotic it was unchanged and in a second it rose slightly. The mean venous oxygen saturation fell from 66 per cent to 55 per cent. These changes are statistically significant ($p < 0.05$).

There was no significant change in the serum levels of sodium, chloride, potassium, or magnesium.

The serum SGOT concentration was measured in 3 control patients and 3 cirrhotic patients before and after fatiguing exercise (100 "power grips"). No significant changes were detected.

DISCUSSION

Cirrhotic patients are particularly apt to develop ammonia intoxication for a number of reasons. First, the functional capacity of the cirrhotic liver

TABLE 4. EFFECT OF EXERCISE ON VENOUS pH, OXYGEN SATURATION AND SERUM ELECTROLYTES

Measurement	*No. of Patients*	*Mean and standard deviation*		
		Resting	*After exercise*	*Difference*
Venous pH	9	7.40 ± 0.03	7.28 ± 0.06	-0.12 ± 0.08
Venous O_2 saturation (vol. per 100 ml)	8	66 ± 12	55 ± 9	- 11 ± 12
Venous CO_2 (mEq./L.)	9	23 ± 2.1	25.1 ± 2.7	2.1 ± 1.1
Serum sodium (mEq./L.)	9	135.8 ± 5.6	137.9 ± 5.2	2.1 ± 2.2
Serum chloride (mEq./L.)	9	100.0 ± 7.3	99.5 ± 6.7	-0.5 ± 1.4
Serum potassium (mEq./L.)	9	4.3 ± 0.55	4.4 ± 0.46	0.1 ± 0.02
Serum magnesium (mEq./L.)	7	1.7 ± 0.2	1.7 ± 0.2	0 ± 0

to remove ammonia and to synthesize urea may be diminished. Secondly, portal-systemic anastamoses permit much of the portal venous blood, which has a high ammonia content, to bypass the liver and to increase the systemic blood ammonia concentration. In addition, some studies have suggested that the removal of ammonia by the peripheral tissues is less efficient in cirrhotics than in noncirrhotic subjects.[29] This may be related to the presence of peripheral arteriovenous shunts which diminish ammonia extraction by the peripheral tissues.[14, 28]

The present investigation indicates that ammonia metabolism in cirrhotic patients may differ from normal subjects in still another manner. Although all subjects liberated ammonia from exercising muscle, cirrhotic patients appeared to release larger amounts after lesser degrees of muscle activity than did noncirrhotics. This was clearly observed with mild to moderate amounts of muscular exertion. After fatiguing exercise, however, both cirrhotic and noncirrhotic subjects exhibited an equally large and prolonged release of ammonia into the venous blood. The local nature of this libera-

tion of ammonia was established by the absence of simultaneous elevations of ammonia in either arterial blood or venous blood draining the resting limb.

Our knowledge of the role of peripheral tissues in ammonia metabolism is based on a relatively few simultaneous measurements of arterial and venous ammonia levels. It has been observed that in the resting, fasting state the peripheral arterial and venous ammonia concentrations are equal or show a small uptake by the peripheral tissues.[4, 21] When arterial ammonia levels are elevated, the arteriovenous ammonia difference increases and may constitute as much as 40 per cent of the arterial level.[3, 4, 29] It has been postulated that this removal of ammonia by the peripheral tissues, presumably muscle, prevents elevations of systemic ammonia levels which are toxic to the central nervous system.[20–23, 34]

The explanation for the greater release of ammonia after relatively mild muscular activity in cirrhotic patients is not clear, but may be indirectly related to the extraction of ammonia by the peripheral tissues. Cirrhotic patients frequently have higher blood ammonia levels than noncirrhotics, and may, by virtue of continued uptake of ammonia by the peripheral tissues, accumulate ammonia in their muscles. The "early" release of ammonia after mild exercise may thus reflect relative saturation of the muscle with ammonia, and may not necessarily involve the same biochemical reactions as the major liberation of ammonia, which follows severe exercise in both cirrhotic and noncirrhotic subjects.

Although the exact sequence of the reactions is not firmly established, adenosine triphosphate (ATP) appears to serve as the source of energy for muscular activity. In the resynthesis of ATP from ADP, adenylic acid (AMP) is formed, and is, in turn, deaminated to inosinic acid. Embden and Parnas, and their co-workers, believed that the ammonia liberated during and after exercise was derived from the deamination of adenylic acid.[18, 31] The significance of this reaction, which does not appear to be important in energy transfer, is unknown.

During muscular exercise many other local metabolic changes take place, including increased oxygen utilization, increased carbon dioxide formation and an increase in hydrogen ion concentration. Although each of these metabolic alterations has been associated with changes in the blood ammonia concentration, it is unlikely that any of these phenomena, per se, is responsible for the increase in venous ammonia levels after exercise.

The pH gradient between the intra- and extracellular fluids has recently been shown to be a major factor in the movement of ammonia across cell membranes.[5, 26] Could a change in the pH gradient account for the increase in venous ammonia values after exercise?

Normally, the pH of the intracellular fluid of muscle is lower than that of extracellular fluid.[28] In the presence of extracellular acidosis there is a decrease in this pH gradient. This favors the passage of ammonia from the intracellular to the extracellular fluid, resulting in an increase in the blood ammonia concentration.[5, 28] Conversely, extracellular alkalosis which increases the pH gradient is associated with the movement of ammonia into the cells and a decrease in the blood ammonia concentration.[5, 18] Fatiguing muscular exercise, which produces a profound local intracellular acidosis[30] also increases the pH gradient. Such a gradient would result in a decrease in blood ammonia concentration and consequently could not explain the increase in venous ammonia levels.

Several observations have suggested that blood ammonia concentrations may parallel the degree of oxygen saturation.[14, 15] Could the increased venous ammonia values after exercise be associated with variations in the venous oxygen saturation? This seems unlikely for several reasons. First, the magnitude of the increase in blood ammonia levels after exercise is much greater than that observed with changes in oxygen saturation. Secondly, the direction of change is opposite to that to be expected with changes in oxygen saturation.

There is some evidence to suggest that the blood ammonia concentration rises as the carbon dioxide tension falls *in vitro*.[10, 27] However, the simultaneous rise in both venous ammonia and carbon dioxide after exercise does not support this relationship *in vivo*.

Muscular exercise is accompanied by a local increase in blood flow which would tend to diminish, rather than enhance, an increase in the venous ammonia concentration. Similarly, the shunting of arterial blood through arteriovenous anastamoses cannot explain the rise in venous ammonia levels after exercise since the arterial ammonia levels are much lower than the venous levels.

There is no evidence to indicate that the release of ammonia during exercise is related either to the uptake of ammonia by the peripheral tissues in hyperammonemic states,[3, 4, 29] or to its discharge into the venous blood under certain circumstances.[20, 21, 34] Bessman and his co-authors postulated that in these situations ammonia was reversibly utilized in the synthesis of glutamine from α-ketoglutarate.[3, 4] Dawson and his co-workers have shown that acetazoleamide may interfere with the uptake of ammonia by the peripheral tissues.[11] This drug did not modify the ammonia rise induced by exercise in our studies.

Although the mechanism of the release of ammonia after muscular exercise is not well understood, this phenomenon emphasizes several points of

practical importance. First, the common practice of having the patient repeatedly clench his fist to facilitate the filling of peripheral veins must be assiduously avoided, since as few as 50 fist clenches may cause significant elevations of the venous ammonia concentration. Although some investigators have noted this artifact,[4, 18] it has not been universally recognized. It is of interest that unusually high venous ammonia values in newborn infants were attributed, at least in part, to the physical activity of the infants during the collection of the blood.[8]

Secondly, the importance of bed rest in the management of patients with impending hepatic coma should be further evaluated. Certainly the role of the peripheral tissues in ammonia metabolism deserves further investigation.

SUMMARY

Mild muscular exercise resulted in an increase in the venous ammonia concentration of blood draining the exercising forearm of cirrhotic patients. Identical exercise in normal subjects did not elevate the venous ammonia level.

More severe muscular exercise caused a significantly greater increase in venous ammonia values in cirrhotic patients than in noncirrhotic subjects.

Severe fatiguing exercise resulted in large elevations in the venous ammonia concentration which were approximately equal in both cirrhotic and noncirrhotic subjects. This exercise-induced rise was not modified by the use of acetazoleamide.

These phenomena are of theoretical and practical importance. Further investigations are needed to clarify the mechanism and significance of these observations.

ACKNOWLEDGMENTS

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INDUCTION OF OVULATION IN THE HUMAN WITH HUMAN GONADOTROPINS.* By C. Lee Buxton and Walter Herrmann, Department of Obstetrics and Gynecology, Yale University School of Medicine.

During the 33 years since human chorionic gonadotropin (hereafter to be designated as HCG) was shown to produce ovulation in small mammals, there have been numerous unsatisfactory attempts to stimulate human ovarian activity by various gonadotropin extracts.[3] Recent evidence of successful species specific gonadotropin extract administration in the monkey and human has been presented by van Wagenen and Simpson[4] and by Gemzell.[1]

The extract used by injection in the present series (HPG)** was obtained from human autopsy material, regardless of the age or sex of the subject. This was prepared by extracting the lyophilized pituitary glands

PATIENTS	NO	AGE	URINARY FSH	17 KS	VAG SMEAR	ENDOMET BIOPSY	BBT	2°SEX
1° Amenorrhoea								
Hypopituitary	1	24	<6.5 mu	10-11 mg per 24 hr	<5% Corn	Ø	Flat	Atrophic
Adrenal Cortical Hyperplasia	1	33	<13 mu	60-80 ß cortisone	0 Corn.	Ø	Flat	Masculinized
2° Amenorrhoea								
"Hypothalamic" or Hypopituitary	6	22 to 27	<6.5 mu	9-11 mg per 24 hr	0-5% Corn.	Ø to Inactive Prolif	Flat	Atrophic to Normal

FIG. 1. Summary of endocrine status of eight patients.

with 40 per cent ethanol and precipitating the extractable material with higher concentrations of ethanol. The precipitate was redissolved, dialyzed, lyophilized and assayed. A later batch (used in J.J.) was purified on an ion exchange column, permitting the removal of most of the LH activity and increasing its FSH activity approximately threefold.

Seventeen courses of injections were given to eight patients, following a course of concentrated chorionic gonadotropin. The latter hormone has been unsuccessfully used in humans by many investigators[3] and its inability to induce ovulation seems to be well established. Nevertheless, since it would appear necessary to control a possible psychogenic effect of the

* The authors wish to acknowledge the advice and cooperation of Drs. van Wagenen and Simpson throughout the process of this study.

** HPG was provided through the courtesy of Merck & Co., Rahway, N. J., in cooperation with Dr. Miriam Simpson, Berkeley, California, who was responsible for the original preparation and assaying of the material. A later batch used in Patient J.P. had been prepared by Dr. S. L. Steelman of Merck & Co.

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present therapy, particularly in patients suffering from hypothalamic amenorrhea, it was felt that the present design would control this aspect and also eliminate HCG alone as the substance responsible for ovulation, should this occur. The patients were evaluated by the means of basal body temperature charts, endometrial biopsies, vaginal smears, and in one case by culdoscopy. They have all been amenorrheic, either primarily or from three to six years. They had various diagnoses, as designated in Figure 1, and all had had thorough endocrine investigations.

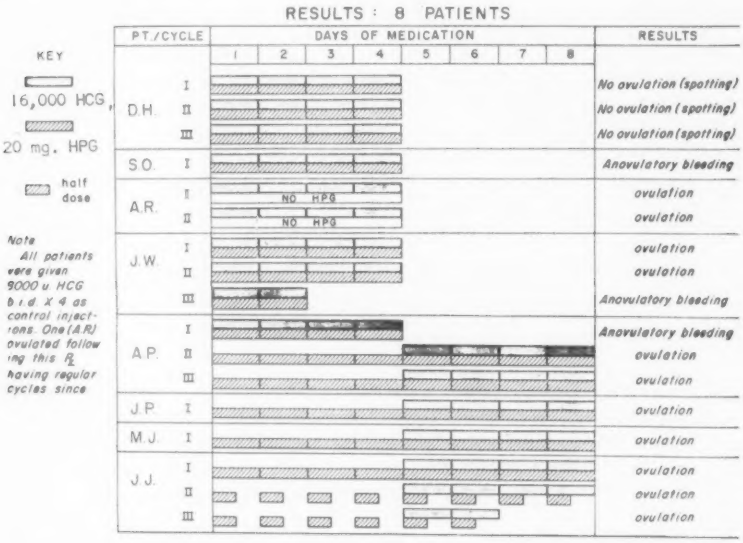

FIG. 2. Result of treatment of eight patients with human pituitary extract and human chorionic gonadotropin.

As is seen in Figure 2, one patient (A.R.) after three years of amenorrhea, ovulated and menstruated following each of the two courses of HCG alone and, as a matter of fact, has continued to menstruate and ovulate since, a result quite different from all of the others. The remaining patients failed to ovulate or menstruate following HCG therapy alone and they therefore were considered appropriate cases to be treated with the combination of HPG and HCG, using a technique and dosage schedule as originally designed by van Wagenen and Simpson for the monkey and adapted to the human on a weight by weight basis. Several variations of therapy were tried at first, before establishing what was considered to be the most satisfactory method. This proved to be the priming of the follicle by administration of 10 mg. of HPG, b.i.d. for four days, followed by four days of administration of HPG, 10 mg., plus HCG, 8,000 units b.i.d. As the more

potent extract became available (see above, Patient J.J.) an attempt was made to decrease the number of injections and amount of hormone given.

The results are apparent in Figure 2. The presence of ovulation was established by the typical changes in the basal body temperature, as well as by secretory transformation of the endometrium. In one patient (A.P.) formerly diagnosed as anorexia nervosa, further documentation was obtained through direct inspection of the ovaries by culdoscopy. This disclosed an enlargement of the ovaries to what was judged to be about three times the normal size, with at least three areas observed in each ovary that were raised above the surface; three were about 1 cm. in diameter and dark purplish or bluish in color, each having at the apex a hemorrhagic stoma. This latter finding is quite similar to the observation of van Wagenen and Simpson in the monkey and of Gemzell in the human.

In summary, seven amenorrheic women were given 15 series of injections of human pituitary material, consisting of human pituitary extract (HPG) combined in part with chorionic gonadotropin (HCG). There were two failures of therapy; one was a patient diagnosed as congenital adrenal hyperplasia, age 33, who had had, at the age of 11, a resection of ovarian tissue and subsequent prolonged treatment with various steriods, primarily of the cortisone series. The other patient responded to the therapy by uterine bleeding, but without evidence of ovulation. She was diagnosed as a Chiari-Frommel syndrome (amenorrhea, galactorrhea, low estrogen). However, she did not receive the full course of therapy, which had proved to be successful in subsequent cases. The other patients, amenorrheic primarily, or for from three to six years, responded to therapy by uterine bleeding from secretory endometrium and had basal body temperature charts characteristic of progestational activity. It was therefore assumed that they had ovulated. One patient, who was subjected to culdoscopy, appeared to have at least three ovulation points in each ovary. These findings are in agreement with the experiments of van Wagenen and Simpson in *Macaca mulatta* and of Gemzell in the human.

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An Observation Concerning the Mechanism of Renal Cyst Formation.*

By Lawrence R. Freedman, *Department of Internal Medicine, Yale University School of Medicine.*

The present report describes the experimental production of renal cysts following localized thermal injury of the papilla (or low medulla) of the normal rabbit kidney.

MATERIALS AND METHODS

A technique of producing intrarenal obstruction in the rabbit has been described in detail elsewhere.[1] In brief, it consists of the insertion of a wire through the surface of the kidney into the papilla or low medulla. Cautery current is then applied, producing a localized thermal injury about 2 mm. in diameter around the tip of the wire. The result is obstruction of a wedge of nephrons. The tubular changes after one week consist of tubular dilatation and atrophy.[2] Glomeruli are not altered. After two to four months, atrophic tubules predominate, but many dilated tubules lined with flattened epithelium can still be found. In some areas there is dilatation of Bowman's capsule to approximately twice its normal diameter.

A modification of this procedure was employed in a group of rabbits in order to produce renal insufficiency. Three burns were placed in the papilla of the left kidney of eight animals. After two to four months the kidneys were removed for histological and bacteriological study and blood samples obtained for the determination of urea nitrogen (BUN).

RESULTS

Seven of the eight injured kidneys showed a variety of scars similar to those found following a single medullary burn and characterized predominantly by severe focal renal atrophy (Fig. 1). The eighth kidney was enlarged rather than contracted and on section had the appearance of the cut surface of a grapefruit (Fig. 2). Microscopically this kidney was seen to consist of a network of greatly distended tubules with recognizable cortex limited to a rim beneath the capsule (Fig. 3). There was a large scar occupying the area where one would have expected to find papilla. Areas of bone formation were seen within this papillary scar. Culture of half of this kidney was sterile. The other kidney of this animal had been removed one week prior to examination and was normal. The BUN determined at the time of sacrifice was 84 mg.%, indicating that this kidney retained some function, since complete loss of functioning renal tissue in a rabbit is associated with death in about three days. Other kidneys showed gross and microscopic evidence of severe atrophy, but had areas within them which were obviously intermediate stages (Fig. 4) of the extreme example of cyst formation illustrated in Figure 3. Culture of one half of each kidney removed was sterile in all instances.

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FIG. 1. Cut surface of rabbit kidney two months after the placement of three cautery burns in the medulla showing atrophic scars.

FIG. 2. Cut surface of rabbit kidney two months after the placement of three cautery burns in the medulla showing diffuse cystic change of the tubules.

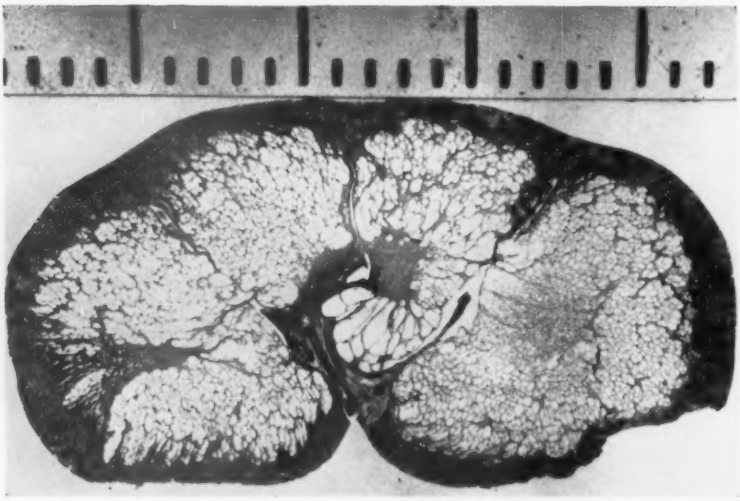

FIG. 3. Microscopic view of section of kidney shown in Figure 3. Each division equals 2 mm.

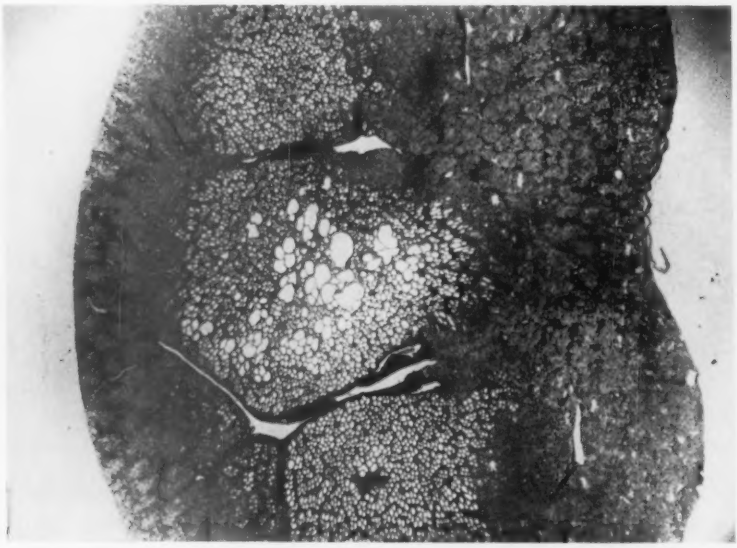

FIG. 4. Low power microscopic view of kidney two months after the placement of three cautery burns in the medulla showing atrophy as the predominant change with some localized cyst formation. (4.5x)

DISCUSSION

Injury to the renal papilla of the rabbit kidney results in two types of change involving the renal tubules: atrophy and cyst formation. Atrophy is the most common lesion, although, examples of localized cyst formation were seen scattered in a random fashion within the obstructed areas. Cyst formation involving the entire kidney was seen once and this kidney resembled very closely the type of polycystic kidney seen in infants.[2] Both the atrophic and cystic kidneys were capable of limited function.

These observations are thought to be of interest for two reasons. The first relates to the theories of production of renal cysts. Whatever the mechanism may be in man, in the rabbit it would appear that damage to the terminal portion of a normal nephron may result in extreme cystic dilatation. This cystic nephron is probably capable of limited function. Indeed, function has also been demonstrated in cystic nephrons in man.[3]

The second point of interest is that a single injury may result in either atrophy or dilatation of nephrons, changes which superficially appear to be quite dissimilar. The fact that they might result from the same type of damage however, raises the interesting possibility that in man the factors leading to the development of polycystic kidney and renal atrophy (hypoplasia) may be related. Some support for this argument is obtained from the observation that cyst formation is quite common in hypoplastic human kidneys[1] just as it was in the atrophic rabbit kidneys described above. It remains for future study to determine the conditions which decide the type of response to be exhibited by specific nephrons following an obstructive lesion.

SUMMARY

Thermal injury of the papilla of normal rabbit kidneys as a rule results in severe atrophy of nephrons with occasional examples of isolated cyst formation. A single instance of complete cystic transformation of the kidney was produced. This kidney was demonstrated to have limited function.

Whatever the cause of renal cyst formation in man, such cysts may be produced in the rabbit by injury to the terminal portion of normal nephrons. It is of interest that injury to the renal papilla may result either in severe atrophy or extensive dilatation of the nephrons tributory to the obstruction. The suggestion is offered that this may have a bearing on the knowledge of the pathogenesis of cyst formation sometimes encountered in human hypoplastic kidneys.

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BOOK REVIEWS

DERMATOLOGIC MEDICATIONS. By M. R. Lerner and A. B. Lerner. Chicago, The Yearbook Publisher, 1960. 208 pp. $6.00.

The renaissance in dermatology which is being led by a small group of research-trained clinical dermatologists has produced much new basic scientific knowledge and a distinct improvement in patient care. The achievements of the authors of the present volume place them in the forefront of the new cutaneous medicine.

Dermatologic Medications, although a second edition, is essentially a completely new and much needed book. In it can be found practically every therapeutic agent of value in dermatology. With great skill based on experience and scholarship the Lerners succinctly describe useful drugs whether of ancient empiric origin or the latest tour de force of the steroid chemist. The indispensable art of topical therapy and the pertinent knowledge of modern pharmacology are skillfully combined to make a volume of great practical value.

One of the important features of the book is the inclusion of the structural chemical formula of each drug where known. The formulae have been grouped and drawn to emphasize the underlying similarities between many drugs in common use. Clinicians, including nondermatologists, will find here much needed help in the selection of a drug unrelated to the one to which the patient is sensitive, or in the selection of a truly different tranquilizer or antihistamine, not merely a different trade name.

This volume reflects the year 1960, not only in its inclusion of the latest drugs but also in its terse communicative style. Nothing of value has been omitted: the use of ultraviolet light or of medicated baths; detailed instructions for treating acne or psoriasis; the background for choosing steroids or tranquilizers; all in simple clear language and a time-saving format. In this reviewer's opinion, Lerner and Lerner's *Dermatologic Medications* will be on every dermatologist's desk and he doubts that many other clinicians will be able to pass it up if they spend a few moments perusing it.

HARVEY BLANK

PROGRESS IN THE BIOLOGICAL SCIENCES IN RELATION TO DERMATOLOGY. Arthur Rook, Ed. Cambridge, Cambridge University Press, 1960. 456 pp. $15.00.

A series of lectures presented in September 1958 at the Post-Graduate Medical School of the University of Cambridge constitutes this book. The course was intended "to bridge the gap between the physician in clinical practice and the scientist carrying out research."

The skin is different things to different people: a framework for nerves, blood vessels, eccrine glands, sebaceous glands, and melanocytes; a tissue which synthesizes keratin, collagen, lipid and melanin; an organ which by its location is suited for experimental carcinogenesis, and so on. This is at once the strength of dermatology as a field for investigation and the weak-

ness of a volume that attempts to construct too many bridges to too many of these interesting sites of investigation.

The first section is concerned with melanin and is well done, especially the chapter by J. I. Harris on the chemistry of MSH, which is really an abbreviated primer on peptide "amino-acid sequence analysis." Brunet's chapter on melanogenesis is interesting and contains, in a note added in proof, the first reference to the work of Seiji who has separated melanin granules from mitochondria by gradient density centrifugation and found no succinic oxidase activity in the former. Thus there is very little reason to further consider the melanin granules as altered mitochondria. The section on cutaneous innervation contains in concise from many of the remarkable studies of G. Weddell who is familiar to dermatologists of this country through his appearances at the American Academy of Dermatology and the Brown University Symposia on the Biology of Skin. Dr. Weddell's investigation of sensory localization leads to surprising conclusions. A single hair of the rabbit ear is served by the axons of two to twenty (average 6) neurons. Since 100,000 hairs are served by only 6,000 neurons, there is necessarily an enormous overlap. Further, different axons of a single neuron differ in diameter and thus in conduction velocity. The picture emerging is not one of direct correspondence of one hair to one neuron but of showers of impulses with a characteristic time pattern which serves to identify the hair stimulated. This implies the existence of a central decoding mechanism more elegant than formerly postulated.

There is a section on radiation with an excellent chapter by J. S. Mitchell on mechanisms of radiation therapy. There remain sections on histochemistry, bacteriology and mycology, psychophysiological mechanisms, comparative medicine, inflammation, pharmacology, immunology, and carcinogenesis. Most of these are methodical treatments of average merit; some are sparse in content. It is regrettable that the lecture by E. F. Gale on the bacterial cell could not have been included.

One is left with the impression that the course did not quite justify a volume although there were occasional excellent contributions.

JOSEPH S. McGUIRE

FALLOUT, A STUDY OF SUPERBOMBS, STRONTIUM 90, AND SURVIVAL. Edited by John M. Fowler, with a foreword by Adlai E. Stevenson. New York, Basic Books, Inc., 1960. 235 pp. $5.50.

This book gives a balanced and understandable account of nuclear weapons and of the multitude of problems they have created for mankind. For the most part, the approach is factual and informative. "Oddly enough," writes Adlai Stevenson in the foreword, "this sober survey of facts is encouraging rather than otherwise. Realities lose some of their terror when we face up to them." One reason for this is evident from the later chapters. Should nuclear war come, those who escape immediate injury from atomic bombs will have another hazard to face: fallout. Their chance of survival may

depend upon their knowledge of fallout and their skill in minimizing personal exposure during the days and weeks following.

It may help us to know and understand these hazards, but the reader will also find a great deal of general scientific interest and much good reading. The book takes the form of a series of chapters. Three have been written by the editor and the rest by experts in the subjects treated. Although this has led to some unevenness in the chapters, the book is, on the whole, interesting, coherent, and easy to read. It is addressed to the reader without specialized scientific knowledge. There is an introduction to nuclear fission and the workings of atomic bombs and the later and more powerful thermo-nuclear bombs. Among the interesting things learned from fallout are the movements of the air masses in the stratosphere made evident by following the world wide distribution of the radioactivity from atomic bomb tests. There is a concise chapter on the levels of fallout in our foods and which have become deposited in our bones and tissues. Then there is an excellent chapter on human genetics and estimates of the effects of radiation on future generations. Much is known of the effects of acute radiation on man from various accidents that have occured in atomic energy plants. Finally, there are realistic chapters on national survival in nuclear war and common sense precautions for personal safety.

This is one book that should appeal to a wide public and can be confidently recommended. It presents the gist of the effects likely to result from fallout in peace and in nuclear war; moreover, it makes interesting reading while it avoids the emotionalism and exaggeration that has sometimes clouded discussion of fallout from atom bomb tests.

PAUL HOWARD FLANDERS

BIOCHEMISTRY OF HUMAN GENETICS. G. E. W. Wolstenholme and C. M. O'Connor, Eds. Boston, Little, Brown and Company, 1959. 347 pp. $9.50.

This book is a collection of papers presented at a Ciba Foundation Symposium held in Naples, Italy, during May 1959. While it is by no means a comprehensive review of the biochemistry of human genetics, the 29 distinguished participants represent a diverse sampling of the active workers in this burgeoning science, and consequently most of the promising new techniques upon which the recent advances in this field have been based are illustrated by the presentations.

During the past decade the enzyme chemist and the geneticist have continued to enjoy a mutually rewarding association. The biochemical elucidation of rare, genetically determined disorders has contributed greatly to our understanding of normal as well as pathological human physiology. Three recent additions to the growing list of enzyme deficiency states are discussed in this Symposium: primaquine sensitivity by B. Childs and W. H. Zinkham, cholinesterase types by W. Kalow, and galactosemia by H. M. Kalcker.

Dramatic advances have been made in the field of protein genetics through the application of the technique of protein fingerprinting. J. A. Hunt and

V. M. Ingraham and H. A. Itano, S. J. Singer and E. Robinson summarize these results in papers on the structure of normal and abnormal human hemoglobins. Of no less interest has been the discovery of inherited variations in human serum proteins by means of two dimensional electrophoretic techniques as described in a paper by O. Smithies and G. E. Connell on the serum haptoglobins and transferrins.

Immunochemistry has long been a cornerstone of human genetics. The fact that this will continue to be a productive approach is attested in articles by W. T. J. Morgan, by W. M. Watkins, and by R. Ceppellini on human blood group substances and by R. Grubb on hereditary gamma globulin groups in man.

One of the most interesting sections of the book is the panel discussion on tissue culture. It will be of interest to see to what extent the potentials and limitations of this promising new technique will be realized in the future.

Other participants in the Symposium include L. S. Penrose, J. Lederberg, J. V. Neel, H. Kalmus, H. Harris, and H. Eagle. Since the book is multi-authored, the clarity and detail of the presentations is quite variable. The over-all effect, however, is to give an exciting glimpse into a fascinating and rapidly expanding field of human knowledge.

WALTER E. NANCE

SEX DIFFERENTIATION AND DEVELOPMENT. Proceedings of a Symposium held at the Royal Society of Medicine, Wimpole Street, London, on 10 and 11 April 1958. Edited by C. R. Austin. Memoirs of the Society for Endocrinology No. 7. Cambridge, University Press. x, 198 pp., illus. $8.50.

The latest memoir of the Society for Endocrinology presents the papers and discussions of a 1958 symposium on selected aspects of comparative and human sex morphology. It is gratifying that discussions of bacteria and invertebrate forms are included. Several authors examine the important question of genetic and hormonal controls of sex differentiation. The possibility of regulating the sex ratio at conception is considered, and so are the origin and grafting of ovarian germ cells and endocrine tissue, nuclear chromatin, failure of normal sexual differentiation, secondary manifestations of sexuality, and other important topics. As is not unusual in a rapidly developing field, terminology occasionally presents a problem. (A separate conference essaying to standardize confused terms in the field of reproduction could be really valuable—conference organizers please take note!) Discussions of the papers, bibliographies, and an adequate index are included. Printing and binding are good.

Naturally no single symposium can include reviews of all aspects of such a broad topic. The papers which were presented, however, are authoritative, clear, and useful.

THOMAS R. FORBES

THORACIC SURGERY BEFORE THE 20TH CENTURY. By Lew A. Hochberg. New York, Vantage Press, Inc., 1960. 858 pp. $15.00.

In this book entitled *Thoracic Surgery Before the 20th Century,* Dr. Lew A. Hochberg has pictured the development of this art during its hundreds of formative years. In most instances, the author has gone to original references and translations for the material to fill this volume of 858 pages. The reader is brought chronologically through the 18th century in the first 186 pages, the remainder of the book being devoted chiefly to the 19th century in a subject-oriented pattern. An extensive bibliography is included.

Although virtually complete and certainly authoritative, the physical make-up of this book makes neither for smooth reading nor ready reference in review. The multiplicity of direct quotations—many of them merely case reports and descriptions of individual, but not particularly contributory, operative techniques—makes it difficult for the reader to retain the central theme of the author, and many times the Gestalt is lost. The intervening narrative is, for the most part, interesting, but its worth is frequently lost by fragmentation. The intriguing story of Dr. John B. Murphy beginning on page 392 is the best of these bridges, and its three-page length qualifies it as the longest bit of narrative, unbroken by quotation, found in the book. More such glimpses through the partly open door would have been welcome.

An especially valuable section is entitled "Non-surgical Contributions to the Advancement of Thoracic Surgery." Such related topics as antisepsis, anesthesia, and x-ray are discussed, together with pertinent historical facets of each. Of especial interest was the reproduction of the "first x-ray taken by Roentgen." This print of a hand in the positive is crude indeed by present day standards, but it taxes the imagination only slightly to realize what an important addition to the physicians' armamentarium this was to become.

One interesting aspect of the direct quotations is the insight provided thereby into the medical thinking of the times, especially as related to judgments of the future based on knowledge of the present. Representative outlooks of the 1890's are expressed by Milton, an obscure English surgeon working in Cairo, Egypt, and by Billroth, "one of the most progressive and enlightened pillars of surgery of his day." The former, with rare vision and astute judgment based on his own experimental work and the works of others, believed that "extirpation of the whole lung may some time or other be classed amongst established operations," and, apropos of heart surgery, that "it requires no great stretch of fancy to imagine the possibility of plastic operations in some of its valvular lesions." The latter, in a far less hopeful vein, wrote that "The surgeon who would attempt suture of a wound of the heart should lose the respect of his colleagues." Sir Stephen Paget went so far as to say, in 1896, that "Surgery of the heart has probably reached the limits set by nature." This only serves to illustrate the fact, implied in several instances in the book, that the important contributions of obscure men may easily escape from view, whereas errors in judgment or prophecy of more prominent persons are not infrequently overlooked.

A book of this type contains many facts and much interesting information and speculation. It is of virtually encyclopedic breadth. I take issue

only with Dr. Hochberg's method of presentation, which the reader may find tiring.

JAMES B. D. MARK

GLAUCOMA, Transactions of the Fourth Conference. Newell, Ed. New York, Josiah Macy, Jr., Foundation, 1960. 257 pp. $8.00.

This is a lively presentation of material which is a must for those working in research on glaucoma. It presents a picture of the frontier of the work by the pioneers who are exploring it. Attention is drawn to the difficulties of fixation of eyes, the variations in interpretation of normal eyes, and how a decision is reached as to what is normal and what is artefact. Against this background, the pathology of the human glaucomatous eye is discussed.

The main feature of this conference is that a new approach to the etiology of glaucoma seems to be evolving, based on research work done by Smelser and Pappas. The implications of this work were really not discussed as the concept of a ciliary epithelium which functions for absorption as well as secretion of aqueous is new, even to conference members.

The reviewer finds the give and take of the conference excellent for calling attention to important aspects of the discussion and differences of opinion, as the material is presented. There is relatively little time wasted on semantics, and digressions from the subject are relatively infrequent and of only brief duration.

The pictures alone show the tremendous advances made, in both light and electron microscopy, in the last decade or so, in the study of the ciliary body and trabeculum. The captions under many of the illustrations contain terms which show that an entirely new nomenclature has had to be developed to describe what is found by these new and improved methods.

The technical nature of the material presented makes this book of limited interest to the clinical ophthalmologist, as there is nothing in it to help him decide how to handle this or that particular case. It is, however, a relatively easy to read presentation of research data for those who are trying to keep abreast of the changing picture of glaucoma.

LOUISE G. LOVEKIN

THE NEUROCHEMISTRY OF NUCLEOTIDES AND AMINO ACIDS. Roscoe D. Brady and Donald B. Tower, Eds. New York, John Wiley and Sons, 1960. xii, pp. $10.00.

This book results from a symposium sponsored by the National Institute of Neurological Diseases and Blindness and held in Philadelphia in April of 1958. The book was published in the summer of 1960. In the field of neurochemistry which has experienced a rapid upsurge of activity in recent years, this two-year delay relegates the "new information" contained in this monograph to ancient history: the chief value of the book essentially lies in reviews on the neurochemistry of nucleotides and amino acids. To this end the book admirably achieves its purpose, especially with respect to the discussion on the biochemistry of the nucleotides. Most of the discussants on

the neurochemistry of amino acids have given their viewpoints in numerous other symposia and, in the opinion of the reviewer, they are long overdue back at the laboratory.

JACK R. COOPER

THE ETIOLOGY OF SCHIZOPHRENIA. Don D. Jackson, Ed. New York, Basic Books, 1960. 447 pp. $7.50.

Psychiatry has been called the art of practising a science which does not exist. This is true at least in part because, although many excellent psychiatric studies are published, the literature is generally not reviewed and synthesized. Psychiatry has no review journal. This book fulfills a specific need, in that it critically reviews the literature on the etiology of schizophrenia.

Book discusses the genetic aspects of schizophrenia, and Abood and Heath argue for a chemical approach to mental disease. Balanced against this is a brilliant critical review of the literature on the genetics of schizophrenia by Jackson. He questions "the assumption that seems to be widely made that there is overwhelming factual evidence for a strong genetic component in the etiology of schizophrenia." Although he concludes, "it seems likely that hereditary factors do play a part in at least some of the schizophrenias," he shows that the genetic evidence is neither overwhelming nor factual and that, indeed, much remains to be done in this important area. Kety critically reviews the evidence for some of the biochemical theories, showing many of the sources of error and artifact. Winder provides an interesting, thorough review of the psychological studies on schizophrenia, and Clousen reports some interesting work on the sociology of schizophrenia. These four papers are particularly important because of their discussion of methodology.

Bowen reports a study in which "the family unit is regarded as a single organism and the patient is seen as that part of the family organism through which the overt symptoms of psychosis are expressed." Bowen showed that some families of schizophrenics who were crazy enough to allow themselves to be hospitalized with their schizophrenic child did indeed show significant psychopathology. That is, parents who allow themselves to be hospitalized with their children for a relatively long period of time may not be a representative sample of schizophrenic parents.

Litz reports an intensive naturalistic study of schizophrenics and their families. Boatman and his group advance the hypothesis that "the etiology of the psychotic disease of children is entirely psychogenic," and therefore report their experience in the treatment of one hundred pre-adolescent schizophrenic children by psychotherapy. They feel that twenty of these children showed improvement in the course of psychotherapeutic work.

McReynolds proposes "that the primary cause of schizophrenia is an excessively high level of unassimilated precepts." Weakland proposes a communication theory of schizophrenia. Certainly schizophrenics, like most people, have two arms and two legs, some unassimilated precepts, and receive contradictory communications. That is to say, schizophrenics are human. To show that any psychological factor causes schizophrenia, one

must not only show that it is present in excess in schizophrenics, and not in normals, but furthermore to elucidate concretely the mechanism involved.

It is interesting to note that in the early chapters of this book on the genetic, biochemical, psychological, and sociological approaches, critical reviews are included. No such review is included in the psychodynamic-family dynamic theories of schizophrenia.

Since this book provides a critical and often brilliant review of most of the literature on etiology of schizophrenia, it is a valuable supplement to the encyclopedic review of schizophrenia by Bellak. It should be read by anyone interested in the etiology of schizophrenia.

JOHN M. DAVIS

PSYCHOPHYSIOLOGICAL REACTIONS TO NOVEL STIMULI: MEASUREMENT, ADAPTATION AND RELATIONSHIP OF PSYCHOLOGICAL AND PHYSIOLOGICAL VARIABLES IN THE NORMAL HUMAN. By Roscoe A. Dykman, William G. Reese, Charles R. Galbrecht, and Peggy J. Thomasson. *Annals of the New York Academy of Sciences, vol. 79,* art. 3, pp. 43-107. New York, 1959. $2.50.

This monograph reports a single empirical study of forty male junior medical students who had their skin resistance, heart rate, and respiration monitored during (1) a rest period of fifteen minutes, (2) a period of eighteen minutes during which they were stimulated by five second, eight hundred cps, sixty dv, tones given at one minute intervals, and (3) a question period of eleven minutes in which subjects were asked questions and instructed to think about the questions but not to answer them out loud. Some of the authors' more interesting findings are as follows: (1) "autonomic responses to serially presented stimuli rapidly diminishes to a relatively constant level." (This has been found in a multitude of studies in the Russian literature.) (2) "The magnitude of the autonomic response is dependent upon the initial level of functioning; in general, the higher the initial level, the smaller the response." (This provides a further documentation of Wilder's rule.) (3) "An individual's reaction in one autonomic system cannot be predicted from his reaction in another." (This point cross-validates studies of Lacey and his co-workers.) A sophisticated, closely written, and highly mathematical discussion of autonomic quantification is provided. Personality correlates of autonomic reactivity are elucidated. This is a carefully done, empirical study cross-validating some basic studies in autonomic reactivity.

JOHN M. DAVIS

PUBLIC HEALTH IN THE TOWN OF BOSTON 1630-1822. By J. B. Blake. Cambridge, Harvard University Press, 1959. 275 pp. $6.50.

John Blake has written a definitive book. No one will have to write another work on public health in the first two centuries of Bostonian history, for this book presents what surely must be the complete story.

By the nature of its subject, the volume is largely concerned with prevention of communicable disease. The most important single event discussed was the introduction of smallpox inoculation in 1721, a topic which possesses a "perennial fascination," as the author puts it, and to which he devotes an important chapter. Three appendices contain valuable statistics.

Blake has constructed his treatise on solid foundations, for he has obviously searched out the most original of sources. Moreover, he has thoroughly documented his statements. The valuable chapter just referred to, "The Inoculation Controversy 1721-1722," occupies 22 pages and stands on 62 footnotes many of which contain two or more titles. Each chapter is similarly supported.

This monograph is more than a medical history. It is as well a valuable contribution to American colonial history, and good, new books on this topic are all too few.

F. G. K.

THE MERCK INDEX, 7TH EDITION. P. G. Stecher, Ed. Rahway, N. J., Merck & Co., Inc., 1960. xii, 1641 pp. $12.00.

After eight years Merck and Company has issued a new, greatly enlarged, edition of their chemical index. A revised and expanded cross-reference system covering ten thousand individual substances makes this book irreplaceable for any chemically oriented life-scientist. Pertinent physical and chemical data are given for all compounds; in addition, structural formulae and significant relevant references are provided for many of the organic molecules. The substances listed range from complex enzyme preparations like pancreatin, to well-defined inorganic compounds. A large appendix section deals with a multitude of subjects, such as biological units, saturated and isotonic solutions, specific gravities, refractive indexes, chromatographic adsorbents, radio-active isotopes, logarithmic tables, and even Greek and Russian alphabets.

This is clearly the choice single reference volume dealing with the chemistry of life. The appendices make it even more valuable and serve to place it on a par with *The Handbook of Chemistry and Physics.* Dr. Stecher and Merck and Company should be lauded on the immense effort involved in preparing their book. Besides this, it is published at cost, still a further professional service by the company.

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